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ERRATA AND AUTHORS' EMENDATIONS

Page 1, lines 25 and 29, page 14, lines 1 and 6, and page 28, eighth line from bottom, "U. S. 875" should be "U. S. 785."

Page 50, fourth line from bottom, "and" should be "of."

Page 96, fourth line from bottom, "(4r)" should be "(14r)."

Page 98, line 3, "(4.5)" should be inserted after "temperature."

Page 118, Table 10, experiments 11, 12, in column 11 headed "Maximum," footnote reference "(*a*)" should be "(*b*)"; experiment 8, in column 11, "3" should be "34," and in column 12 headed "Index for optimum temperature" "35" should be "3."

Page 128, after literature citation (9), "WITT, A. W." should be inserted after "KNIGHT, R. C."

Page 131, line 33, insert between "*giganteum*" and "in" the following, "on creeping bent in putting greens of golf courses."

Page 263, line 19, "Raos" should be "Rosa."

Pages 358–359, Table 1, and pages 360–361, Table 2, last column headed "Period of survival," "Grams" should be "Days."

Page 500, third line from bottom, insert "(Fig. 2, C.)" after "crystals."

Page 518, twelfth line from bottom, omit "C."

Page 552, Table 2, third line from bottom, "Protein (×6.25)" should be "Protein (N×6.25)."

Page 554, Table 4, omit superscript "*a*" after "Ratio of" and its corresponding footnote.

Page 610, line 4, reference to Reimer should be (*23*), not (*22, 23*).

Page 613, line 9, and page 614, line 13, reference to Reimer should be (*22*), not (*22, 23*).

Page 681, twenty-sixth line from bottom, under column headed "Plants erect at harvest," "84.1" should be "81.4."

Page 724, fourth line from bottom, "species" should be "specimens."

Page 739, line 4, (*10*) should be (*9*); line 17, (*10*) should be (*9*) and (*9*) should be (*10*).

Page 748, Figure 1 should be inverted.

Page 761, nineteenth line from bottom, "two" should be "three."

Page 796, Table 1, fourth column, under "Moyock" the "Do." opposite "Hahto" should be opposite "Haberlandt."

Page 799, Table 3, eighth column, under "Average coefficient" opposite "Columbia," "0.9" should be "0.09."

Page 896, line 15, "1,000" should be "100."

Page 963, eighth line from bottom, "gm." should be "c. c."

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DEVELOPMENT OF FLOWER AND SEED OF SOME VARIETIES OF SUGAR CANE[1]

By ERNST ARTSCHWAGER, *Associate Pathologist*, E. W. BRANDES, *Principal Pathologist in Charge*, and RUTH COLVIN STARRETT, *Assistant Cytologist, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The investigation presented in this paper is part of a comprehensive study of the anatomy, the ontogeny, and the cytology of sugar cane, undertaken primarily as a basis for further research in diverse fields including taxonomy, physiology, pathology, and genetics. The Office of Sugar Plants now has projects in all these fields, and it is proposed by the present series of studies to lay a foundation that will be useful in advancing them. A previous publication which discussed the anatomy of the vegetative organs of sugar cane (*1*)[2] was based on a critical examination of a single variety, Louisiana Purple (Zwart Cheribon), of the species *Saccharum officinarum*, to which belongs the large group of thick-stemmed or "noble" cane varieties, preferred, where they can be successfully grown, by all commercial cane planters. It is recognized that owing to the diverse forms of Saccharum it is unsafe to generalize on the basis of a study of one variety or a limited number of varieties. Therefore the logical plan is to study a single variety in detail, and to follow this with comparative studies of the minute anatomy of selected parts, which will doubtless reveal differences that may be useful in helping to separate and identify varieties. Studies of the inflorescence are especially difficult and narrowly circumscribed, in so far as the range of selection of varieties is concerned, on account of irregularity in flowering of sugar cane, which takes place only when environmental conditions are exceptionally favorable and, moreover, on account of the frequently encountered degeneracy or abortive development of the male and female organs. The varieties used in the present study, U. S. 875 and U. S. 1694,[3] were those available in sufficient quantity in the United States sugar-plant field experiments at Canal Point, Fla., when the investigation was begun in January, 1927. The variety U. S. 875 is from the F_2 progeny of a cross between *Saccharum officinarum* (Zwart Cheribon variety) and *S. spontaneum*, a wild species; and U. S. 1694 is from the F_2 progeny of a cross between *S. officinarum* (Zwart Cheribon variety) and *S. barberi* (Chunnee variety).

According to Jeswiet (*12*, *13*), the genus Saccharum embraces four species: *S. spontaneum*, *S. sinense*, *S. barberi*, and *S. officinarum*.

[1] Received for publication Aug. 10, 1928; issued July, 1929. This paper reports the second of a series of investigations of the anatomy of sugar cane. The first was entitled "Anatomy of the Vegetative Organs of Sugar Cane" (*1*).

[2] Reference is made by number (italic) to "Literature cited," p. 29.

[3] U. S. = United States Department of Agriculture seedling.

Another species, provisionally named *S. robustum*,[4] was found in New Guinea by Doctor Jeswiet when he accompanied the United States Department of Agriculture airplane expedition to the interior of that island in 1928. In the following key, which has been translated from Jeswiet's original paper, are given the characters on which the separation of the species is founded:

KEY TO THE SPECIES OF SACCHARUM

A. Main axis of inflorescence and cluster axes with long hairs. Glumes always 4. Lodicules either ciliate or not. If the spikelets of the same pair do not flower simultaneously, the pedicellate one always blooms first. Culms green, grayish green, greenish bronze, ivory, or white.
   1. Lodicules ciliate, long subterranean runners present, growing wild ---------- *Saccharum spontaneum.*
   2. Lodicules not ciliate. Subterranean runners short. Sugar-producing, cultivated plants.
      a. Leaves broad (to 50 mm.). Long cane species, nodes all fusiform, greenish bronze (among others, Uba cane)---------- *Saccharum sinense* Roxb. amend. Jeswiet.
      b. Leaves narrow, short inconspicuous cane species. Nodes usually cylindrical, grayish green, white, or ivory. Rather limited to British India (among others, Chunnee cane)----*S. barberi* Jeswiet.

B. Main axis of the inflorescence never having long hairs, often glabrate; rachis nodes glabrate or with very few hairs. Glumes generally 3, sometimes 4. Lodicules not ciliate. If the spikelets of the same pair bloom at different times, the sessile one always is the first. Culms differing in color from pale or dark green to dark yellow, dark red, violet, often striped. Cultivated plant------- *Saccharum officinarum.*
   1. Fourth glume present. Vigorous plants with low sugar percentage. Types: Among others, Fidji, Ardjoeno, Green Coerman (Corven Duitsch), New Guinea.
   2. Fourth glume wanting. Plants with a general high percentage of sugar. Types: Among others, Cheribon, Batjan, Borneo, Bandjermasia, Preanger.

It will be noted that the separation of species in this key is based on the panicle characters. In seeking to establish a basis for varietal determination it is necessary to make use of the vegetative characters as well. Barber (*2*) in his monumental work on the sugar canes of India considered the characters of stem, leaves, and roots. Various investigators have made use of special characters such as the number and arrangement of the stomata, the number and disposition of root primordia in the root ring, and the character and arrangement of emergences on the buds and leaves; but no system has been found sufficiently practicable to commend itself to general use, and it has ebcome increasingly apparent that the problem is so complicated that accurate determination of cane varieties is a matter for specialists. In the present investigations anatomical characters have been observed to vary sufficiently in the different varieties to be of value in supplementing the morphological characters ordinarily used in attempting to separate varieties.

METHODS

The flower material was fixed in Carnoy's fluid directly in the field. The material was embedded without removing the scales, since it was found that in young material the scales are not sufficiently hard to interfere with the cutting, and in older material the presence of the scales, by preventing too rapid dehydration and infiltration with paraffin, resulted in better preservation of the nuclei. Mature seed

[4] The new species *Saccharum robustum* is not distinguishable from *S. officinarum* on the basis of panicle characters thus far studied.

fixed while still in the milk stage could be cut comparatively easily even if embedded in paraffin. Older seed had to be embedded in celloidin. All cytological material was stained with Haidenhain's iron-alum hematoxylin, and histological material with Delafield's hematoxylin, and counterstained with saffranin.

All photomicrographs were taken on Wratten M plates with B–58 and E–22 filters used singly and in combination. The drawings [5] are based on photomicrographs or their enlargements.

THE INFLORESCENCE

The inflorescence of sugar cane forms an open panicle. (Fig. 1, A.) Every species and almost every variety has, according to Jeswiet (*13*), its peculiar type of inflorescence or "arrow." Depending on the type of lateral axes, the arrow will be small or large, broad or narrow, conical or cylindrical. The main axis of the inflorescence is longest in *Saccharum officinarum*, shortest in *S. spontaneum*, and intermediate in *S. sinense*. It is thick, straight, and round at the base, becoming gradually thinner, sinuous, and distinctly grooved higher up. While the inflorescence is still closed, the lateral axes rest in these grooves, permitting the entire arrow to be squeezed into a very compact space.

The main axis carries lateral axes of the first order, and these in turn lateral axes of the second and third orders. The degree of branching decreases toward the top, and the upper part has only whorls of single axes. However, if these axes are branched, the secondary axes are inserted in such a manner that the joints of primary and secondary axes are grown together. (Fig. 2, A.) The lateral axes of the first order are longest and most profusely branched. The lower ones are far apart and arranged in semiwhorls, and the upper ones are close together and form complete whorls. The lateral axes of the second order and all primary axes with the exception of the lower ones begin with a pair of spikelets of which one is sessile and the other stalked. (Fig. 1, B.)

The developing inflorescence remains for a long time inclosed in a tube formed by the sheaths of the upper vegetative leaves. When the floral leaves become externally visible, the cane is spoken of as "boenting," a convenient term adopted from the Malayan language, for which there is no adequate English equivalent. Several weeks after the first indication of boenting, the flag (fig. 1, C) appears, and the inflorescence, which has a silvery hue on account of the silky hairs of the spikelet, is pushed out and expands. At the base of each lateral axis is present a cushionlike swelling, which enables the lateral axes to unfold and the inflorescence to open completely.

ANATOMY OF THE FLORAL AXIS

As a continuation of the apical growing point of the stem, the inflorescence axis shows a similar anatomical structure. A cross section shows numerous vascular bundles embedded in parenchymatous tissue. The central part is devoid of bundles and is often hollow. At the periphery the bundles are so small and so close together that they form a practically solid ring. The vascular tissue is separated from the epidermis by a narrow cortex, the cells of which are thick walled and heavily lignified. The epidermis itself consists of long

[5] Credit is due Mrs. E. Artschwager for preparation of the colored plate and all other drawings in this publication.

narrow cells with sinuous walls and alternating short cells. At the insertion points of the lateral axes there are cushionlike swellings

FIGURE 1.—A, Inflorescence of sugar cane fully expanded; B, lateral axis of inflorescence bearing pairs of spikelets; C, inflorescence in the "flagging" stage

made up mostly of parenchymatous tissue with the vascular elements limited to the abaxial side of the organ. This special modification

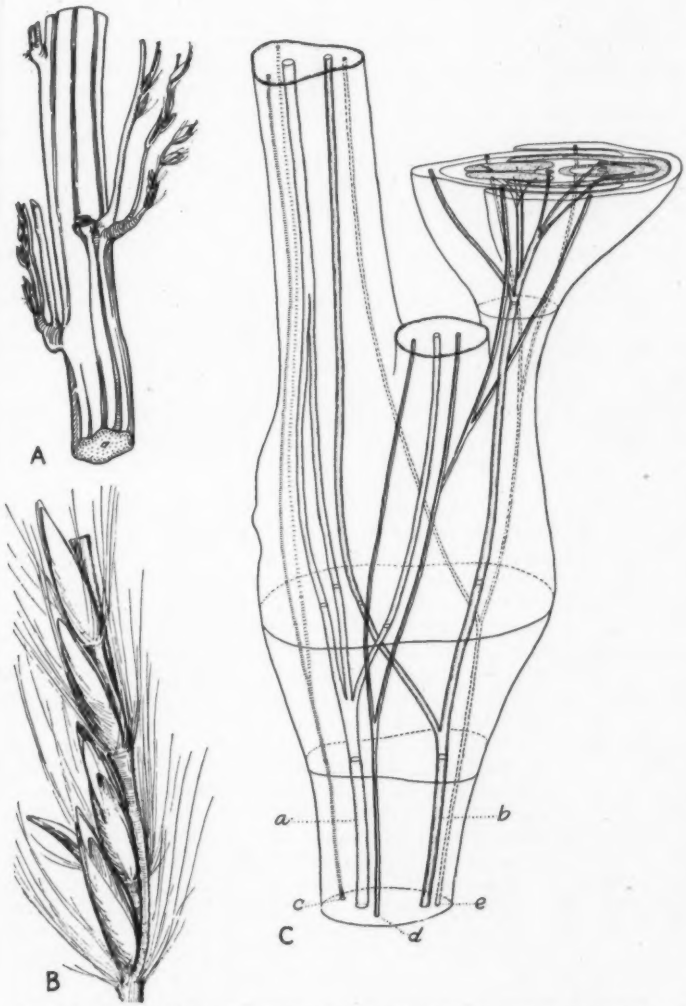


FIGURE 2.—A, Lateral axes arising from the same cushion joint of upper part of main axis. B, Part of rachis of inflorescence. Each rachis articulation bears a pair of spikelets of which one is sessile and the other stalked. C, Diagram illustrating the course of the vascular system of the rachis, the pedicel, and the sessile flower; *a* and *b* represent the two large bundles of the rachis and *c*, *d*, and *e*, the three small peripheral bundles

enables the lateral axes to expand, and at the same time it gives a greater flexibility to the joints and prevents injury during abnormal weather conditions.

The lateral axes of the first order are very much thinner than the main axis. The basal part is flat triangular, becoming more or less terete higher up. Like the main axis, it is traversed by a number of vascular bundles of which the inner ones are large and the peripheral ones small and surrounded by thick-walled lignified parenchyma. The lateral axes of the higher order are more or less flat triangular and possess a varying number of vascular bundles. The rachis, which bears the spikelets and which may be an axis of the first, second, or higher orders, possesses from four to six bundles, of which three are small and the remaining one to three much larger. The stalk of the pedicellate spikelet differs from the rachis in being thinner and possessing only three bundles, of which two are small and the third one much larger.

A transverse section of the rachis below the cushion joint shows a stele of five bundles and from these bundles there pass off a number of traces to supply the floral parts of the sessile flower and of the pedicel. (Fig. 3, A–H.) Above the point of departure of these traces the rachis continues, possessing the number of bundles it contained below.

The rachis joint, where the branching of the bundles occurs, differs from the rachis in being round and possessing a much greater diameter. (Fig. 3, A.) The cells of the parenchyma tissue are small and very short. They have greatly increased, forming a broad band between the more or less centrally located vascular strands and the epidermis. Many cells of the latter have enlarged and grown out into lignified hairs. Above this zone are several tiers of epidermal cells conspicuous on account of their very regular radially elongated form and the dense protoplasmic content. Just beneath the epidermis is found a sclerenchymatous hypodermis one cell wide.

A little distance below the cushion joint the two larger bundles (fig. 2, C, *a* and *b*) break up into three and two strands, respectively (fig. 3, A, B, C, D). Two strands of *a* and one of *b* continue into the next rachis segment, and the third strand of *a* passes out into the pedicel, while the second strand of *b* constitutes the larger part of the vascular system of the sessile flower. Simultaneously with the splitting of the two larger bundles the three small bundles of the rachis also divide. The two halves of each separate more or less and then extend into the various organs, as shown in the accompanying figures.

Frequently the rachis possesses three larger bundles instead of two and occasionally only one. When there are three large bundles, one extends into the next rachis segment without dividing, but if there is only one, two divisions occur, and the resulting three strands extend into the various organs, where they form the larger bundles. The behavior of the small bundles is in all cases more or less identical.

The outer glume of the flower is traversed by four and sometimes five or more bundles, and the inner glume is keeled and contains commonly three vascular strands. The sterile lemma has frequently a midrib, but the fertile palea and the fertile lemma, if present, have no vascular elements. The thick hyaline lodicules each contain a number of procambial strands. Each stamen has a single bundle. The remaining vascular tissue splits into three strands, which traverse the tissue of the ovary wall and converge in the style.

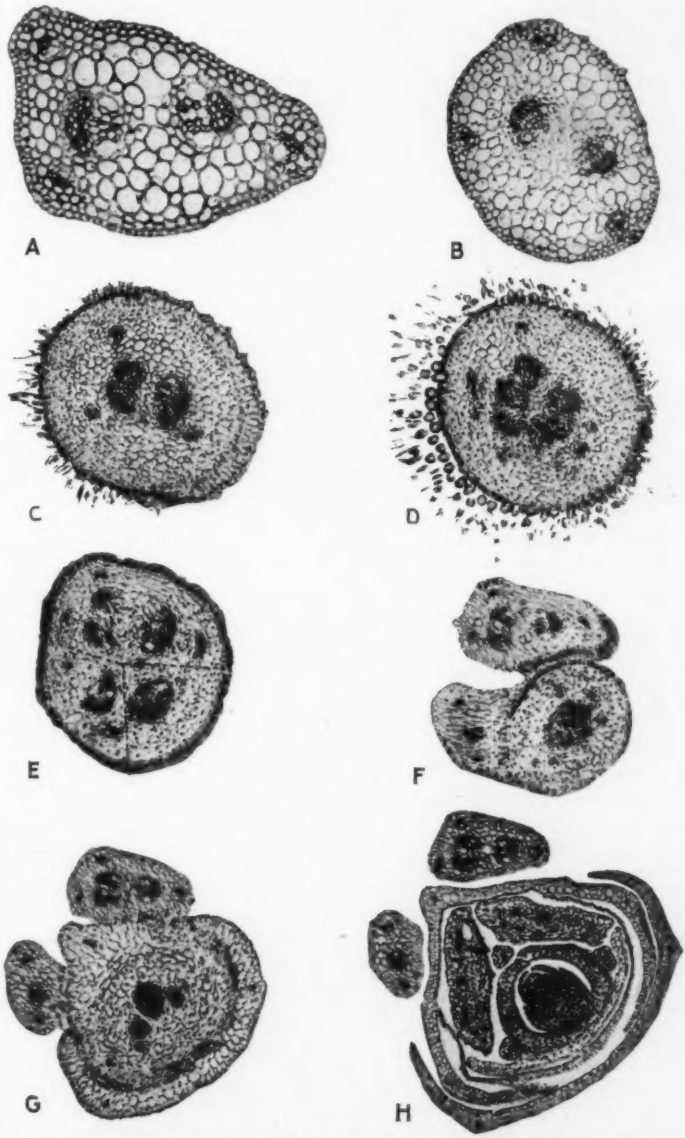


FIGURE 3.—A–H, Series of cross sections through a rachis joint (cf. fig. 2, C), illustrating the course of the vascular system of the rachis, the pedicel, and the sessile flower in the U. S. 875 variety. A, × 165; B–H, × 67

THE SPIKELET

The rachis (fig. 2, B) to which the flowers are attached is articulate and very brittle. Each rachis segment is narrow at the base and broader at the apex, one side of it being more or less convex and the other flattened and slightly concave.

At the nodes of the rachis are alternately placed two spikelets, of which one is sessile and the other stalked. Both spikelets are oblong lanceolate and uniflorous and have tufts of long silky hairs at their bases.

Each flower (pl. 1, A, and fig 4, A) is subtended by two bracts which form the outer and inner glumes. Inside the outer glume is found a sterile lemma (third glume), inclosing in its turn the fertile palea. The fertile lemma (fourth glume) was wanting in the varieties of *Saccharum officinarum* examined, but was present in the hybrids of *S. spontaneum*. The sterile palea is always wanting. At the base of the flower, just inside the inner glume, are found two thick hyaline lodicules. Lastly, the axis bears a whorl of three stamens and the ovary.

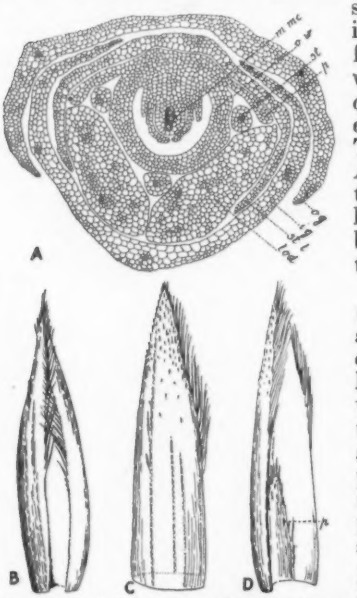
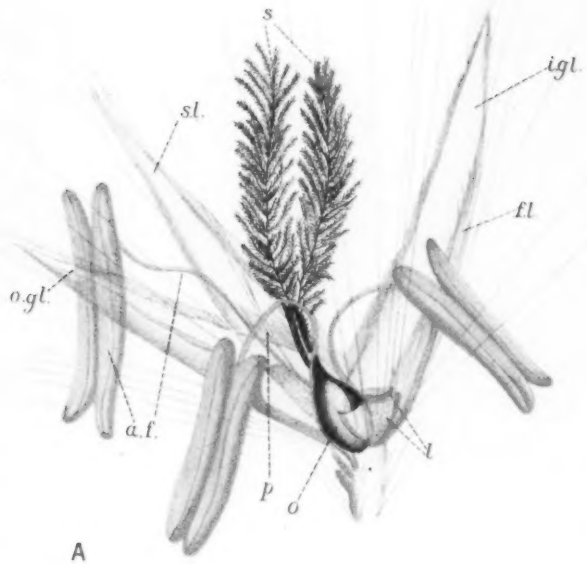


FIGURE 4.—A, Cross section of a young flower of U. S. 1694: *m mc*, Megaspore mother cell; *o w*, ovary wall; *st*, stamen; *p*, palea; *o g*, outer glume; *i g*, inner glume; *st l*, sterile lemma; *lod*, lodicule. × 80. B, Outer glume. C, Inner glume. D, Sterile lemma inclosing the palea (*p*)

The outer glume of the spikelet (fig. 4, B) is bicarinate with slightly arched back and embraces with its edges the inner glume. The two keels and the edges are traversed by vascular bundles, which may extend to the tip of the glume. Occasionally a weakly developed midrib is present. The upper two-thirds of the glume is covered with short pointed hairs. The keels possess stronger hairs than the lamina between, and the edges are covered with lashes composed of long unicellular hairs. From the foot of the glume, the callus, arises a whorl of long silky hairs, several times the length of the spikelet.

The inner glume (fig. 4, C) is, like the outer, lanceolate pointed, but it possesses a well-developed midrib. On either side of the midrib is found a small vascular bundle. The back of the glume is also covered with short, closely adhering hairs, but the lashes along the edges are slightly longer.

The sterile lemma (fig. 4, D) is much more delicate than the two glumes, and it is commonly devoid of vascular bundles. It is a little shorter than the inner glume. The tip is slightly blunt in the varieties examined, but according to Jeswiet (*13*) it can be either blunt or pointed. The edges of the sterile lemma are covered with long lashes, which are especially prominent near the middle. The fertile lemma, which was found only in the *Saccharum spontaneum* hybrids, is a



A

B

A.—Open sugar-cane flower of variety U. S. 759; *i. gl.*, inner glume; *o. gl.*, outer glume; *s. l.*, sterile lemma; *f. l.*, fertile lemma; *p*, palea; *l*, lodicules; *o*, ovary; *s*, stigma; *a.*, anther; *f.*, filament of anther. X15.
B.—Stages in the germination of sugar-cane seed. X15.

narrow scale covered at the tip with hair or lashes and is devoid of vascular bundles.

The fertile palea is also small (fig. 4, D, *p*) and very irregular. Its edges are clothed with long hairs, and a few short hairs are found on the upper surface of the lamina. It is, according to Wilbrink (*18*) and Jeswiet (*13*), very changeable regarding shape and pubescence, but since these characters are constant in different forms, it serves in the study of taxonomic relationships.

The lodicules (fig. 5, E) are very short, more or less wedge shaped, with saucer-shaped hollow tops. They possess short projections on the upper edges, which, in the case of the *Saccharum spontaneum* hybrids, may elongate into longer or shorter lashes. During the time of flowering the lodicules swell greatly and push the glumes wide apart (fig. 5, E) so that the anthers and the pistil can protrude.

THE FLOWER

The sugar-cane flower is hypogynous and simple in structure, consisting of a whorl of three stamens and a single carpel. Often the flowers are imperfect, the ovary being rudimentary and, what is more often the case, the anthers without pollen grains.

The number of stamens is three; one filament is inserted between the lodicules and the other two on the opposite side of the flower, resting against the fertile palea. (Fig. 4, A.) The filaments are white and the anthers yellow and later purple. If they remain yellow or become papery they are invariably sterile. The anthers are bilobed and versatile, the filaments being attached to the connective near the base of the anther lobes. Each lobe contains two loculi (fig. 6, D), and the tissue that is between breaks down at the time of dehiscence. The filament before the opening of the flower is terete. It consists of elongated parenchymatous cells surrounding a small centrally located vascular bundle. When flowering occurs the filament grows very rapidly, thereby becoming irregular in thickness. The tissue of the connective is parenchymatous with a small bundle similar to that in the filament. The epidermis of the lobes is composed of narrow cells covered by a thin cuticle. Next to the epidermis is the endothecium composed of narrow cells with their long axes arranged at right angles to those of the epidermis. Shortly before dehiscence they develop thickening bands.

In the literature on sugar cane, references to the structure of the flower are mostly fragmentary. Of more than passing interest is the contribution of Cobb (*7*).

The gynecium (fig. 5, A) consists of a single carpel with an obovate or obconical ovary round in transverse section and flattened on the ventral side. The walls of the ovary consist of delicate parenchyma cells. Running longitudinally through it are two very fine lateral vascular strands. From the tip of the ovary arise two terminal styles which curve outward when flowering occurs. (Fig. 5, B.) Within the ovary is a single ascending anatropous ovule attached by a broad placenta on the inner ventral surface of the ovary. It possesses two integuments. The outer one, which disintegrates when the seed is ripe, is much shorter than the inner one and never reaches the micropyle. Each integument has a double layer of cells, but in the region above the placenta and at the micropyle it consists, as already shown by Guérin (*10*), of three or four layers.

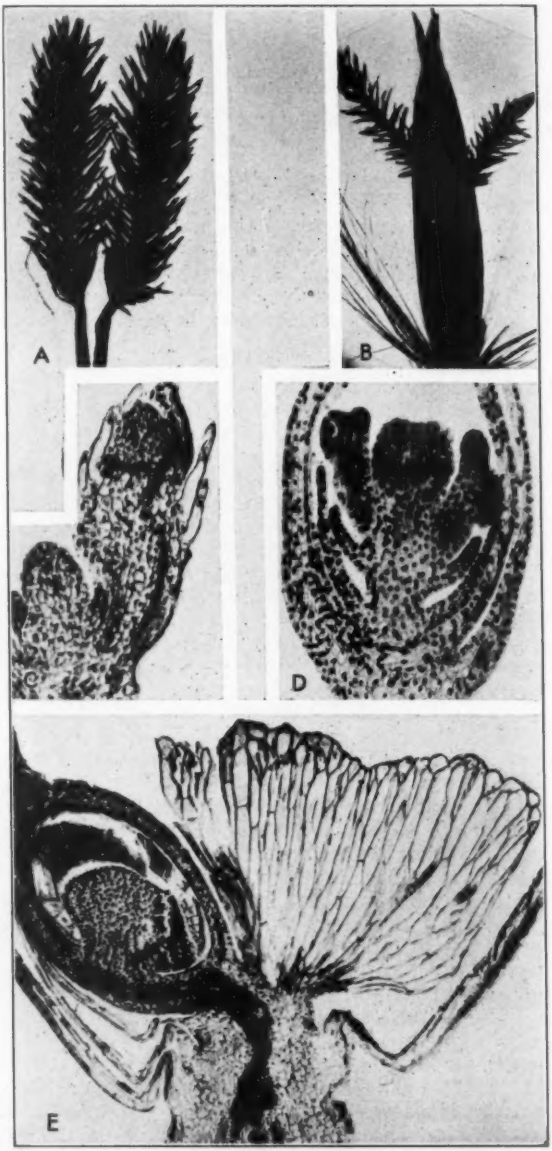


FIGURE 5.—Sugar-cane variety U. S. 1694: A, Gynecium, × 17; B, flower about to open, × 9, showing protruding tips of the style; C, longitudinal section of a pair of young spikelets, × 110, the stalked spikelets being advanced in development over the sessile one; D, longitudinal section of an older spikelet, × 110; E, longitudinal section of an expanded flower of U. S. 1639, × 50, the lodicules having forced the flower open through their ability to swell

DEVELOPMENT OF SPIKELET AND FLOWER

The stalked spikelet starts development before the sessile one. It shows already the developing glumes and hairs, while the sessile spikelet is just beginning to differentiate from the primordium. (Fig. 5, C.) The sessile spikelet develops at the inner side of the

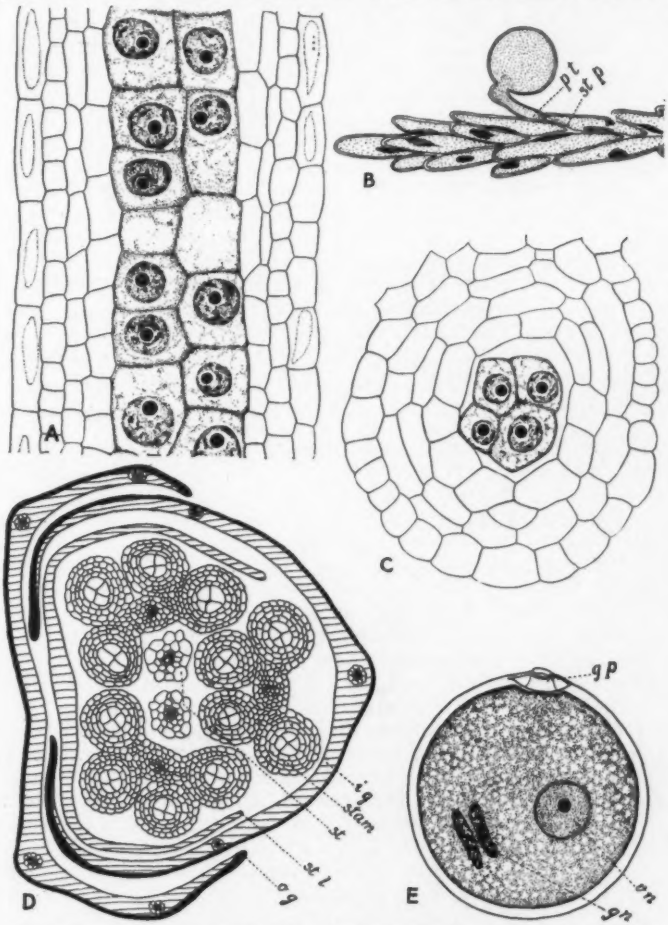


FIGURE 6.—Sugar-cane variety U. S. 1694: A, Longitudinal section of an anther with pollen in mother-cell stage, × 680; B, lateral branch of feathery stigma with germinating pollen grain, × 272; *pt*, pollen tube; *st p*, stigmatic papilla; C, cross section of an anther with pollen in mother-cell stage, × 680; D, cross section of a young flower: *i g*, Inner glume; *stam*, stamen; *st*, style; *st l*, sterile lemma; *o g*, outer glume; × 110. E, mature pollen grain; *v n*, vegetative nucleus; *g n*, generative nuclei; *g p*, germ pore; × 1224

base of the pedicel, and for this reason there are four rows visible at the convex side. (Fig. 2, B.) Only a little of the sessile spikelet is seen at the back side, so that there is a conspicuous dorsiventrality.

A detailed description of the development of the grass flower, especially of the androecium and gynecium, is given by Golinski (*9*)

and Koernicke (*14*), and with it the description of the sugar-cane flower, as given below, tallies. The floral organs and the bracts by which they are inclosed arise in succession as bulging masses of meristem upon the periphery of the primordium, beginning with the lower glume and ending with the carpel. (Fig. 5, D.) Each begins as a ridge of tissue that extends part way around the primordium of the flower. The small fertile palea and the lodicules arise at the same time and from the same rudiment. They are at first triangulate but gradually acquire the oblong shape characteristic of the mature organ. The stamens arise as conical outgrowths around the periphery of the apex of the flower. They develop early, the filament appearing before the lamina of the lower glume is differentiated and while the carpel is still small. A constriction which indicates the beginning of the filament occurs very early at the base of the stamen. The subsequent growth of the stamens until the pollen is ripe is due almost entirely to the elongation of the anthers, the filament growing only immediately before the pollen is shed. The carpel is the last of the floral organs to arise. It appears as a single ridge of tissue on the interior side of the floral axis and gradually encircles the apex of the young flower. Soon after it has completely surrounded the tip of the flower axis, two protuberances appear on the sides and form the beginnings of the stigmas.

MICROSPOROGENESIS

It is not within the compass of this investigation to treat in detail the early stages of karyokinesis, for it would lead to a consideration of the reduction problem in general. The sugar-cane flowers, moreover, are not suited for such a study, since the nuclei are comparatively small and the division figures, especially the early prophase stages, are complex and minute in structure.

Dividing pollen mother cells are found in inflorescenses that have just begun to flag. They appear naturally first at the top of the inflorescence and tips of the lateral axes and progress centripetally and downward as the panicle expands. According to Bremer (*4*), this difference in the appearing of the pollen mother cells at the top and at the base amounts to six days in *Saccharum officinarum*, but to only four days in *S. spontaneum*. The young pollen mother cells are seen in longitudinal sections of the anther (fig. 6, A) in two rows fitting closely into one another and bordered by tapetum cells. In cross sections of the anther one observes usually four pollen mother cells arranged in such a manner that they all touch the tapetum with one of their sides. (Fig. 6, C and D.)

The young pollen mother cells contain only a little chromatin, which is present in the form of small grains. As the cells increase in size the chromatin grains enlarge, become more numerous, and appear connected with one another by fine threads. Gradually the nuclear mass assumes a unilateral position and the chromatin threads exhibit the characteristic, but in this case indistinct, prosynaptic pairing. Contraction increases until the spireme shows the typical klumpen. Chromatin threads are then thrown into the nuclear cavity until the whole space becomes filled with the chromatic filament. (Figs. 7, B; 8, A; and 9, A.) The threads of the spireme, being double, appear to be thicker than the prosynaptic ones. The spireme is soon after segmented; the individual parts continue to

shorten and thicken until finally in the diakinetic stage (figs. 8, B, and 9, B) the bivalent chromosomes are formed. The number of paired chromosomes or gemini is very large compared to the size of the nuclear cavity, and it is impossible to count them accurately. Shortly after the formation of the bivalents the spindle fibers begin

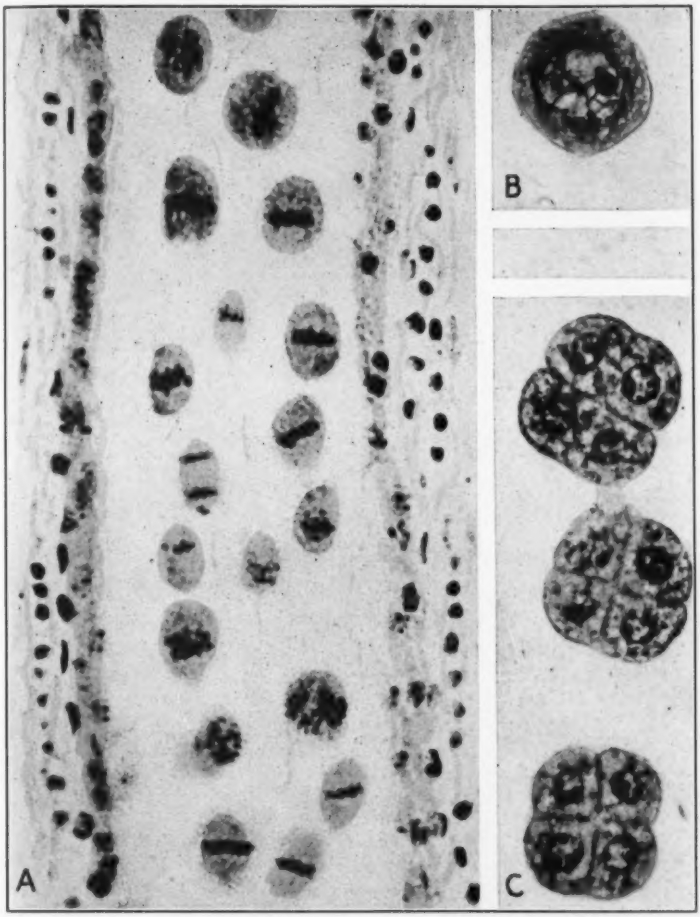


FIGURE 7.—Sugar-cane variety U. S. 1694: A, Longitudinal section of anther with pollen in metaphase of the heterotypic mitosis, × 520; B, prophase stage of the heterotypic mitosis, × 840; C, pollen tetrads, × 750

to differentiate, while the nuclear wall disappears. The shortening and thickening of the gemini continues until the metaphase when the chromosomes become almost isodiametric and deep staining. The chromosomes are now seen on the equatorial plate of the bipolar spindle. (Figs. 7, A and 8, D.) The polar view of the metaphase (figs. 8, C, and 9, C) is especially well suited for the counting of the

chromosomes. It was found that in the U. S. 875 variety the number of chromosomes was 58. Since this variety is a Kassoer seedling, it should, according to Bremer (*4*), possess 68 chromosomes. The smaller number might be accounted for by assuming that the material that was used in this investigation came from a cross between U. S. 875 and another variety, or that the hybrid, by subsequent selfing, suffered a reduction in the number of its chromosomes. In the other

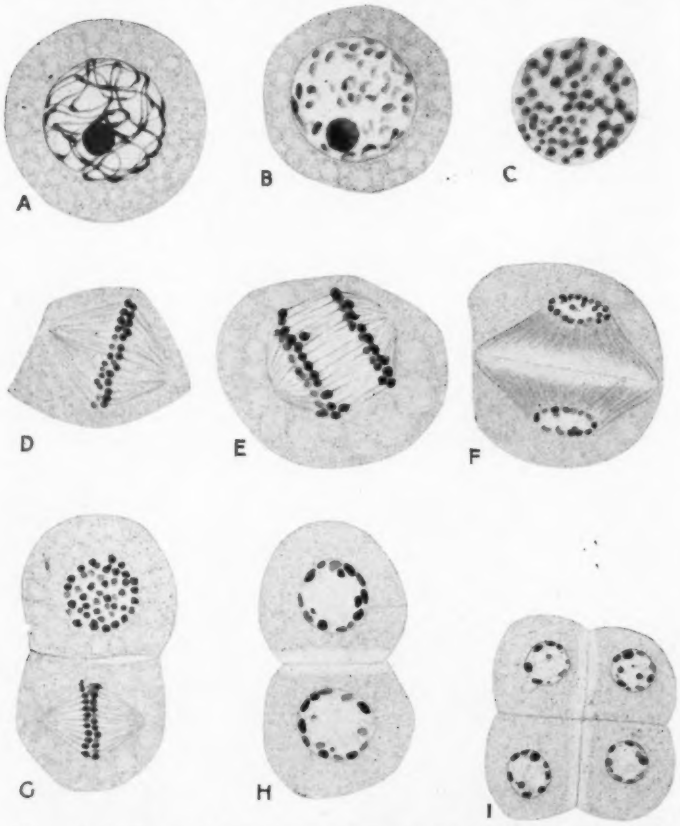


FIGURE 8.—Sugar-cane variety U. S. 785 at various stages in microsporogenesis, × 1140: A, Hollow spireme; B, diakinesis; C, polar view of metaphase; D, metaphase; E, anaphasic separation; F, telophase; G, homotypic metaphase; H, diads; I, tetrads

variety, U. S. 1694, the number of chromosomes counted was 40, and since it is a P. O. J. 213 seedling this number is theoretically correct. The chromosome number of 14 which Franck (*8*) found for *Saccharum officinarum* does not agree with the haploid number of 40 found by Bremer (*4*) which was verified in this investigation.

While metaphase stages persist for a comparatively long time, the anaphasic separation (fig. 8, E) is quickly accomplished and appears to be quite normal, since there was practically no lagging of chromo-

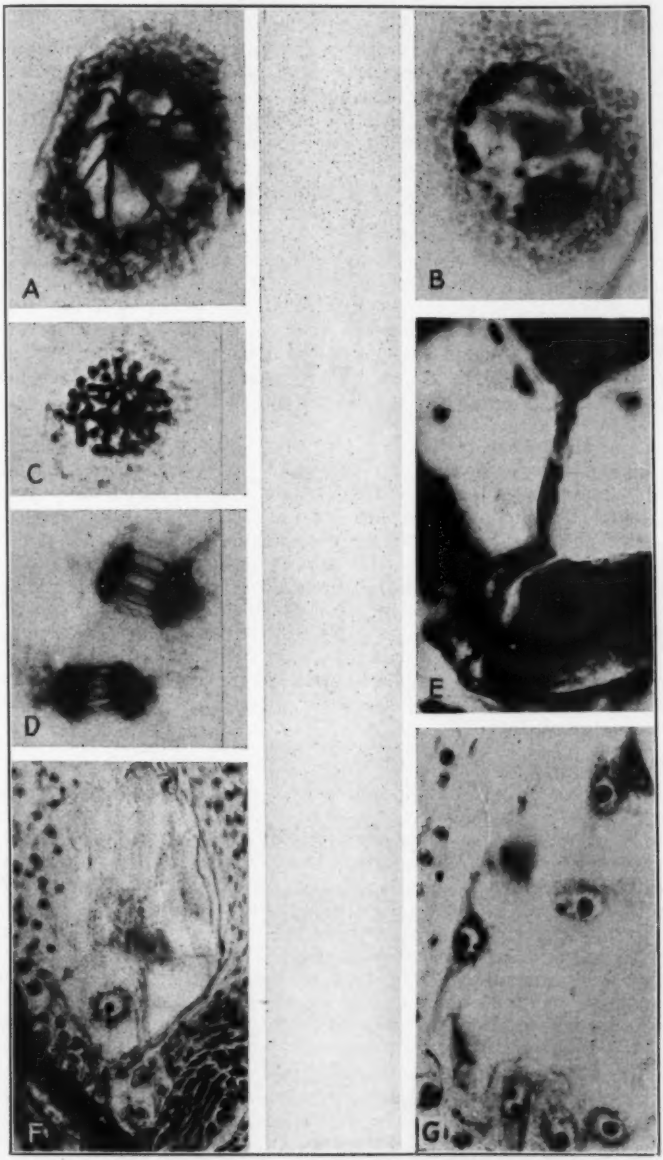


FIGURE 9.—A, Prophase stage (hollow spireme) of the heterotypic mitosis in pollen formation, × 1330; B, diakinesis, × 1330; C, polar view of metaphase of U. S. 875, × 1330; D, anaphase of endosperm nuclei of U. S. 1694, × 740; E, pollen tube penetrating micropyle of U. S. 1694, × 740; F, embryo sac, with fertilized egg and endosperm nucleus in anaphase, U. S. 1694, × 215; G, endosperm nuclei lying free in the embryo sac, U. S. 1694, × 230

somes. At telophase (fig. 8, F) the chromosomes form a dense mass so that their number at this stage can not be ascertained. Just as in other monocotyledons, the two daughter nuclei are separated by a wall. (Fig. 8, H.) There is a short interkinesis during which the chromosomes retain their distinctness. In the homotypic division the nuclear spindles are usually arranged with their longitudinal axes parallel to one another, though occasionally they cross at right angles. (Fig. 8, G.) The chromosomes have the same isodiametric appearance, but they are smaller than those met with in the heterotypic division figures. The tetrad soon breaks up (figs. 8, I, and 7, C) into four microspores, which become invested by a wall that at first is thin and delicate. The extine of the mature pollen grain is thick (fig. 6, E), smooth, and possesses a single pore. It agrees essentially with the figures published by Schacht (*17*). In the protoplasm of the pollen grain are embedded three nuclei. The two generative nuclei are elongated and possess numerous dark-staining chromatin granules. The vegetative nucleus is spherical and has a large spherical nucleolus, but it stains only weakly compared to the generative nuclei.

While the pollen mother cells undergo the two divisions and the pollen is developing, changes occur in the various layers of the anther wall. The cells of the middle layer that have remained small become stretched tangentially and are soon obliterated. The cells of the tapetum are densely filled with protoplasm and contain at first a single nucleus. At about the time the sporogenous cells enter synapsis the nuclei of the tapetum divide and the cells become typically binucleated. At the time of the formation of the tetrads the tapetal cells show signs of disorganization; they separate and the content breaks down and is absorbed by the developing pollen. The cells of the epidermis and of the endothecium enlarge with increase in the size of the anther, and shortly before the dehiscence the cells of the endothecium develop thickening bands.

MACROSPOROGENESIS

The young ovule is atropous and consists first of a hemispherical mass of tissue derived from the morphological apex of the floral axis. Soon, however, as a result of differential regional growth it curves and becomes anatropous. During this change in position of the ovule the ovary increases greatly in size. It is flattened at first, becomes spherical, and finally oblong.

The archesporium is of subepidermal origin. It is wedge shaped (fig. 10, A) and at the time when both integuments of the ovule are initiated it is easily made out by reason of its relatively large size. It occupies over one-third of the nucellus and is eight times the size of the surrounding cells. (Fig. 10, B.) It keeps on enlarging until the ovule has both integuments fully developed. Then it divides rapidly twice, forming a tetrad row of megaspores. The outer three cells are gradually disorganized and remain for a time as deeply staining masses which finally disappear altogether. (Fig. 11, A.) The surviving mother cell enlarges and, pushing apart the cells on the sides of the narrow channel left by the disintegrating megaspores, comes in contact again with the epidermis.

The development of the embryo sac to the 8-celled stage follows the usual course. (Fig. 12, A–E.) The functional megaspore nucleus

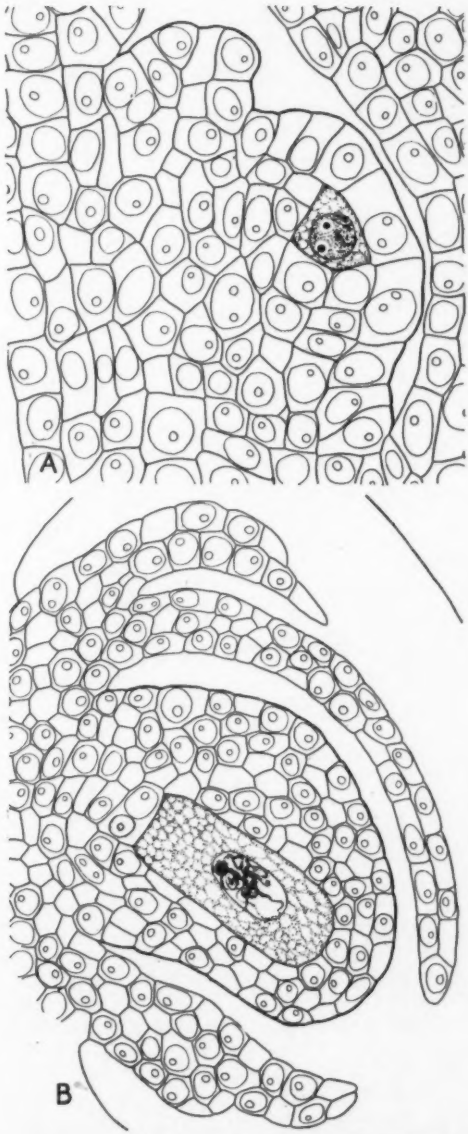


FIGURE 10.—A, Young ovule with megaspore mother cell differentiating, × 650; B, ovule with megaspore mother cell greatly enlarged and integuments already developed, × 650

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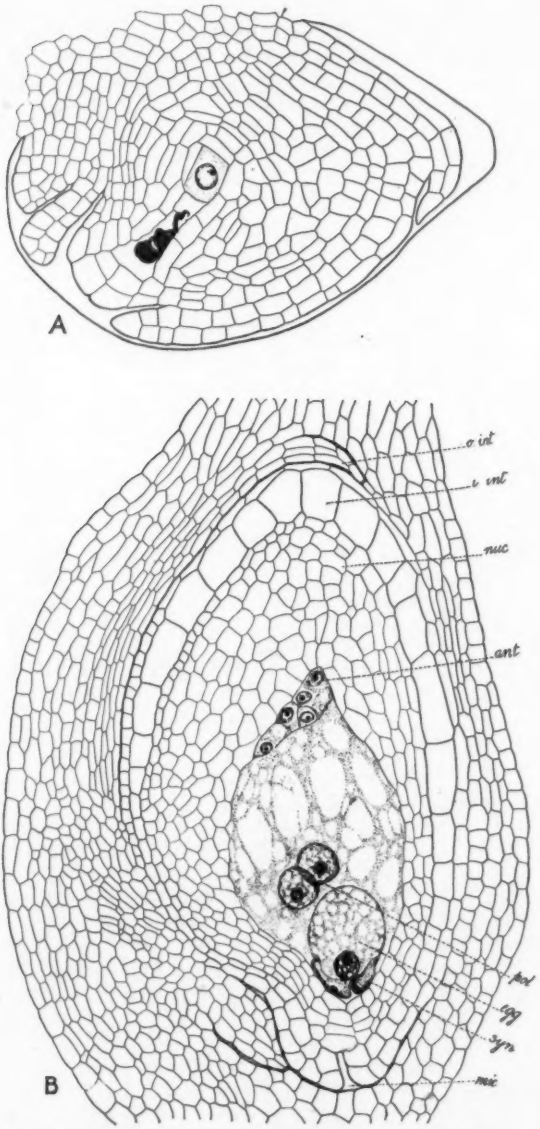

FIGURE 11.—A, Ovule with row of megaspore tetrads of which three are degenerated, × 250; B, ovule with 8-celled embryo sac, × 250: *o int*, Outer integument; *i int*, inner integument; *nuc*, nucellus; *ant*, antipodals; *pol*, polars; *egg*, egg; *syn*, synergids; *mic*, micropyle

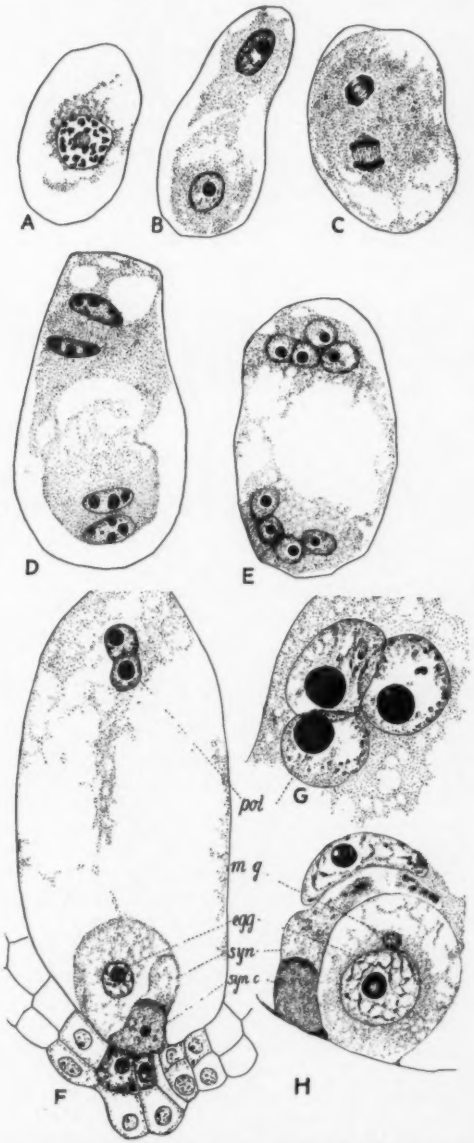

FIGURE 12.—Sugar-cane variety U. S. 1694: A–E, Different stages in the development of the 8-celled embryo sac, × 600. F, An unusual type of embryo sac in which the polars are far removed from the egg, × 288. For legend see Figure 11, B. G, A group of three polar nuclei in mature embryo sac, × 600. H, Fertilization of the egg; the male gamete is seen inside the egg: *m g*, Male gamete; *syn c*, synergidal cap; *syn*, synergid, × 600

divides into two nuclei, which lie one in the micropylar end and the other in the region of the chalaza. The chalazal nucleus divides at right angles, and the upper nucleus divides parallel to the long axis of the embryo sac. The nuclei of each group divide again. The sac now contains eight free nuclei. (Fig. 11, B.) At the micropylar end are two pear-shaped synergids with typical synergidal caps and a broader egg nucleus. The synergids do not readily take the stain, and their nuclei are rarely visible. Near the egg are the two polar nuclei, which remain in close contact with the egg and fuse to form the primary endosperm nucleus just before fertilization. Abnormalities in the development of the polars were observed on two occasions. In one preparation (fig. 12, F) the polar nuclei of an otherwise normal embryo sac were far removed from the egg and practically abut on the antipodals; in another preparation (fig. 12, G) there were observed three well-developed polars instead of two. The three nuclei at the chalazal end of the sac give rise to the antipodal complex. (Fig. 13, A.) The antipodal nuclei divide several times and each nucleus becomes inclosed in a globular mass of protoplasm. The antipodal complex shows in section frequently a characteristic crescent form. The nuclei vary in size (fig. 13, A); they often stain deeply; at other times they appear very faint. The disorganization of the antipodal nuclei begins simultaneously with the formation of the endosperm.

The mature embryo sac is covered at the apex by about two layers of nucellar tissue, the central ones somewhat elongated so that the apex of the nucellus is slightly pointed. (Fig. 9, F.) Within the embryo sac the granular cytoplasm forms an intricate network and is especially dense around the two polar nuclei. A longitudinal sagittal section of the mature embryo sac is shown in Figure 14, A. The sac is narrow in the upper part, gradually broadening toward the region of the micropyle. In sections cut at right angles to the sagittal the sac appears uniformly narrow. (Fig. 14, B.)

While the embryo sac is developing the nucellar tissue becomes disorganized, its disappearance being most rapid in the micropylar region and the dorsal side of the ovule. The epidermis of the nucellus continues for a long time. Its position and the arrangement of the cells render it liable to be mistaken for the aleurone layer before the latter is distinctly separated from the endosperm. Later its cell content becomes disorganized and the layer disintegrates.

ANTHESIS OF FLOWERS AND POLLINATION

The anthesis of the flowers takes place in the early morning hours before daylight in the latitude of Canal Point, Fla., according to Brandes (*3*). Calvino (*5*) confirms this in work conducted at San Manuel, Cuba, and states that meteorological conditions greatly influence the opening time, the dehiscence of the anthers being especially delayed by the presence of dew. Flowers that are about to open are recognized by the protruding pistils from the still closed glumes. (Fig. 5, B.) The filaments of these flowers are meanwhile increasing in length rapidly while the lodicules begin to swell, gradually forcing the glumes apart. The flowers usually remain open for only a short time; the pollen is quickly shed and carried by the wind to the receptive stigmas of the flowers.

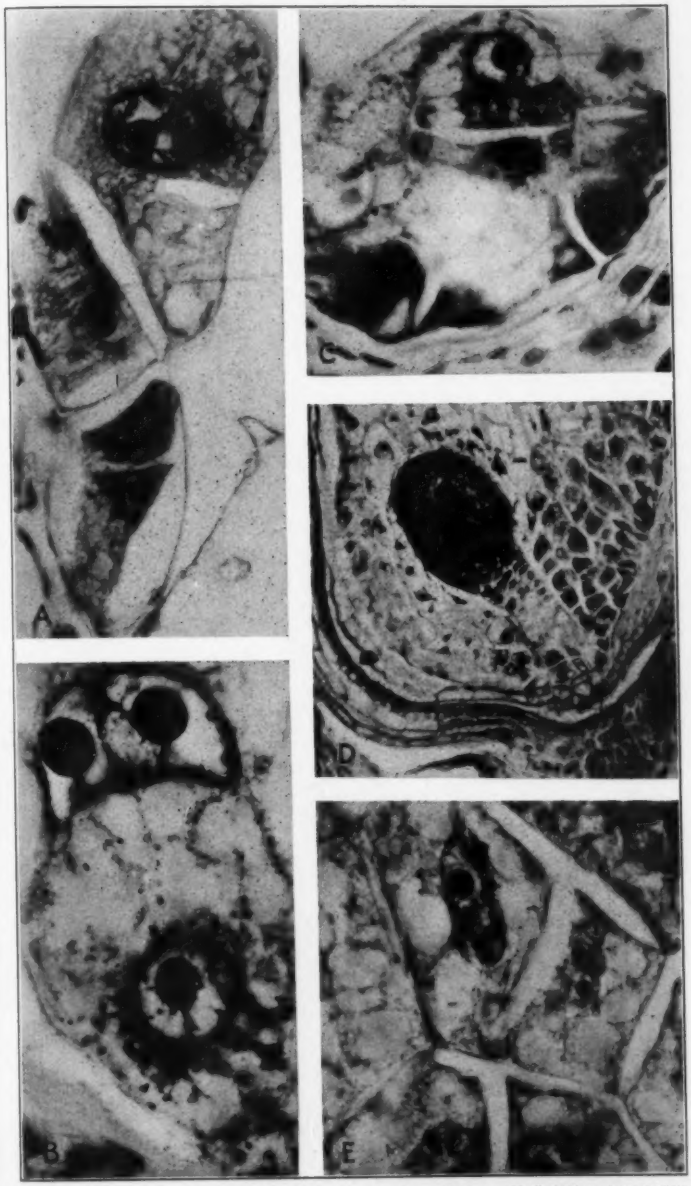

FIGURE 13.—A, Antipodal complex at time of fertilization of the egg, U. S. 1694 variety, × 850; B, egg and polars at time of fertilization, U. S. 1694, × 850; C, 3-celled embryo, U. S. 875, × 850; D, older embryo, U. S. 875, × 220; E, cellular endosperm, U. S. 875, × 850

FERTILIZATION AND DEVELOPMENT OF SEED

The pollen, falling upon the receptive stigmatic papilla, germinates immediately. The pollen tube grows through the papilla (fig. 6, B) into a lateral branch of the stigma and thence downward until it reaches the conductive tissue of the style. Upon entering the ovary cavity it grows between the outer integument and the ovary wall to the micropyle, pushing down between the cells of the nucellus (fig. 9, E), which appear to be injured by its passage. The further history of the pollen tube and the actual discharge of the male nuclei could not be followed in the material that was available for study, yet the presence of the male gametes in the egg cell (fig. 12, H) and the appearance of two nucleoli inside the egg nucleus (fig. 14, C) of another preparation indicate that fertilization has taken place and that the process may be taken as normal.

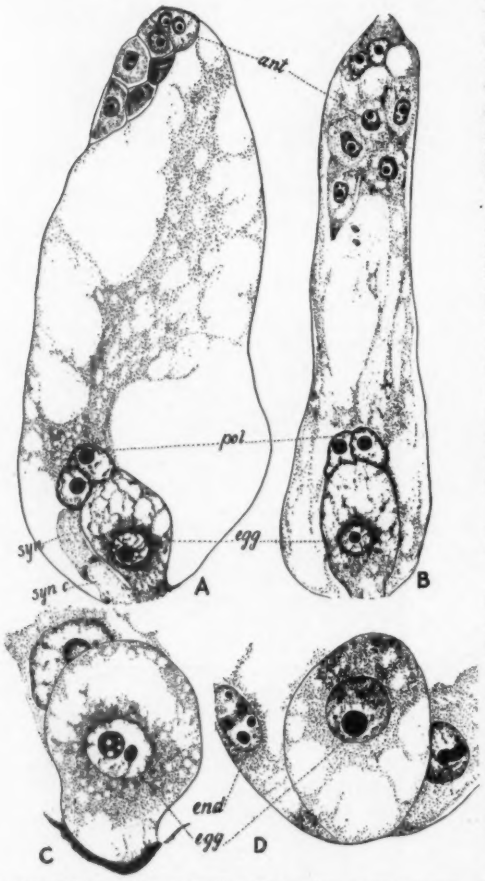

FIGURE 14.—A, Longitudinal sagittal section of embryo sac: *pol*, Polars; *syn*, synergids; *syn c*, synergidal cap, × 208. B, Longitudinal radial section of embryo sac: *pol*, Polars, × 208. C, Egg nucleus with two nucleoli, × 520. D, Fertilized egg at rest surrounded by free endosperm (*end*) nuclei, × 520. For legend see Figure 11, B

The egg enlarges somewhat after fertilization. (Fig. 14, B.) It is pear shaped, sometimes adhering with the narrow end to the micropylar end of the sac and sometimes possessing a broader base of attachment. In this condition it remains until the development of the endosperm is well advanced.

The polar nuclei at the time of fertilization are found in close contact with the egg. (Fig. 13, C, and 14, A, B.) They fuse at the time the egg is fertilized or soon after and at once begin to divide rapidly. (Fig. 14, D.) The first nuclei are seen in the thin protoplasmic lining in different parts of the embryo sac. (Fig. 9, G, and fig. 15.) The nuclei differ

among themselves at all stages in the number of nucleoli which they possess. Division in the nuclei is at first simultaneous, since one observes the same division figures in all the nuclei of the sac. (Fig. 9, D.) With their increase in number they become larger in size, cease dividing, and become inclosed in rather dense masses of protoplasm that later becomes separated by cell walls. (Fig. 13, E.)

The first division of the fertilized egg is transverse and results in the formation of a smaller epibasal and a larger basal cell. (Fig. 15.) The second division occurs in the basal cell and is also transverse. (Fig. 13, B.) This sequence of divisions appears to be common in grasses, as shown by the studies of Nörner (*16*) and Cannon (*6*). The third division takes place in the epibasal cell, bisecting it parallel to the long axis of the embryo sac. The third division, however, may occur first in the cell below the epibasal, or it may occur almost simultaneously in both cells. Since most varieties of sugar cane produce even under favorable conditions only a small percentage of seed, it does not lend itself to embryonic studies. Although embryos in various stages of development were obtained (fig. 13, B, D), it was impossible with the material on hand to follow the sequence of division and to follow out the ontogeny of the different parts of the mature organ. Further work may disclose that failure of the fertilized egg to keep pace with the growth of the ovule and to form a viable seed is due to cessation of growth while yet in the proembryonic stage, or that it results from abnormalities in the reduction division, or unfavorable environmental conditions during the pollination period.

STRUCTURE OF THE SEED AND SEED COAT

The fruit of the sugar cane, like that of other grasses, is a caryopsis. It is narrow, ovate to oblong, and in the region of the embryo there is commonly visible a slight depression. At the proximal end is the scar of the seed, while the distal end often bears the remains of the style. (Pl. 1, B.) When immature the seed has a milky-white color, but when ripe it is a yellowish brown. The seeds vary considerably in size in spite of their minuteness, but it is of interest to note that the decrease in size is caused by a reduction in the amount of endosperm tissue and that the size of the embryo itself is more or less constant. Cobb (*7*) illustrates the structure of a mature seed with an excellent drawing of a cross section near the distal end of the embryo.

A median longitudinal section through the seed (fig. 16) shows, at the proximal end, the relatively large embryo, the position of which is marked externally by a slightly lighter color. The embryo is partly surrounded by endosperm tissue, and both embryo and endosperm are covered by the seed coat. The embryo possesses a short mesocotyl, at the apex of which is the growing point surrounded by rudimentary leaves and inclosed by the coleoptile; at the base is the radicle completely inclosed by the coleorhiza. Almost surrounding these organs is the scutellum. (Fig. 17, B, C.) It is expanded laterally into two wings and extends farther down than the root, from which it is separated by a distinct cleft. The epidermis of the back of the scutellum, which is in contact with the endosperm, consists of more or less columnar epithelial cells which secrete diastase for the conversion of the stored food of the endosperm. The plumule

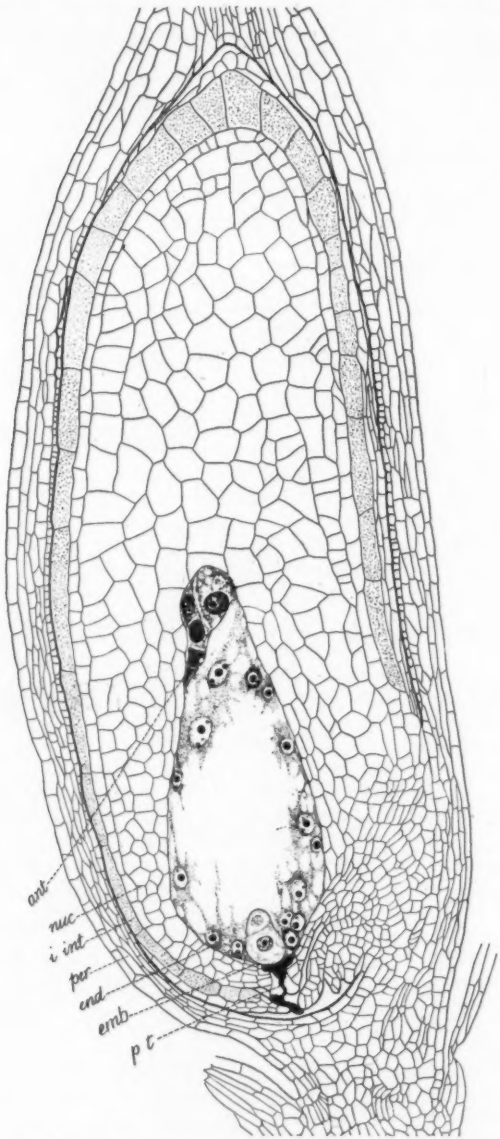

FIGURE 15.—Sugar-cane variety U. S. 1694. Longitudinal sagittal section of ovule with 2-celled embryo and numerous endosperm nuclei: *ant*, Antipodals; *nuc*, nucellus; *i int*, inner integument; *per*, pericarp; *end*, endosperm; *emb*, embryo; *p t*, pollen tube. × 160

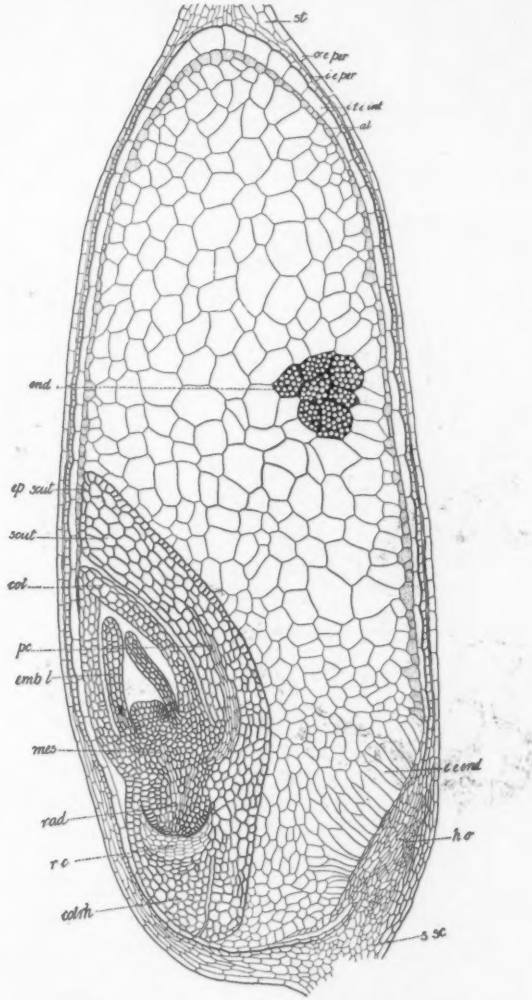

FIGURE 16.—Sugar-cane variety U. S. 1694. Sagittal section of a mature seed and embryo: *st*, Remains of style; *o e per*, outer epidermis of pericarp; *i e per*, inner epidermis of pericarp; *i i i int*, inner layer of inner integument; *al*, aleurone layer; *end*, filled endosperm cells; *e e end*, empty elongated endosperm cells; *h o*, cells of hilar orifice; *s sc*, seed scar; *ep scut*, epithelium of scutellum; *scut*, scutellum; *col*, coleoptile; *p c*, procambium strand; *emb l*, embryonic leaves; *mes*, mesocotyl; *rad*, radicle; *r c*, root cap; *coirh*, coleorhiza. × 75

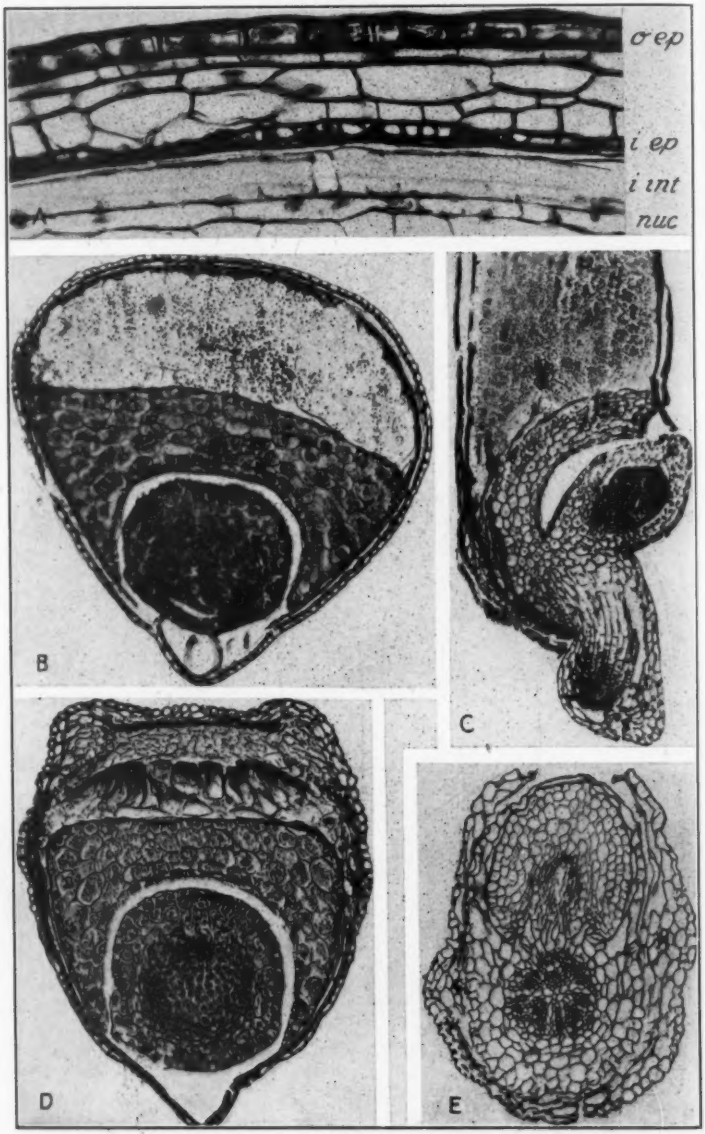

FIGURE 17.—Sugar-cane variety U. S. 1694. A, Ovary wall at time of fertilization: *o ep*, Outer epidermis; *i ep*, inner epidermis; *i int*, inner integument; *nuc*, nucellus, × 420. B, Cross section of mature seed cutting through scutellum and tip of coleoptile, × 110. C, Section through the same seed but at level of radicle, × 110. D, Longitudinal section of germinating seed, × 46. E, Cross section through root region of young seedling, secondary and primary roots are separating, × 70

consists of several rudimentary leaves surrounding the growing point. The radicle is covered by the root cap and is inclosed within the coleorhiza. The latter is a parenchymatous tissue connecting with the mesocotyl and the lower half of the scutellum.

The vascular system is present in the form of procambial strands consisting of elongated thin-walled cells. A broad procambial strand curves outward almost at right angles from the mesocotyl and extends upward in the scutellum. Upon the germination of the seed the procambial cells become differentiated into vascular elements. Simultaneously the epithelial cells elongate slightly and the endosperm cells next to the scutellum become devoid of starch.

Just inside of the seed coat and extending entirely around the embryo and the endosperm except in the region of the hilum and the micropyle is the aleurone layer. It is usually one cell thick, but occasionally several cells are superposed. The individual cells are rectangular in transverse and longitudinal sections and polygonal with rounded corners in surface view. In front of the scutellum the layer consists of smaller cells, while over the plumule and coleorhiza it becomes so thin and shriveled that it is difficult to recognize it as a separate layer. Each cell of the aleurone layer contains an oval nucleus with several nucleoli; the rest of the cell is filled with spherical aleurone grains embedded in an oily cytoplasm. In the region of the hilum and the proximal end of the scutellum lies a light-colored mass of elongated somewhat irregular empty endosperm cells. The endosperm tissue is made up of thin-walled polyhedral cells with those adjoining the aleurone layer smaller than the central ones.

The seed coat of the sugar-cane seed consists of the fused pericarp and the inner integument. The pericarp is made up of several layers of cells, of which only the outer and the inner epidermis are distinguishable. The outer epidermis is slightly undulated and consists of larger rectilinear cells with relatively thin walls. (Fig. 17, A.) The inner epidermal consists of thin-walled tubular cells, which lie parallel to the outer epidermal cells running from base to apex. (Fig. 17, A.) They are circular or broadly elliptical in cross section.

The inner integument forms a single layer of cells, continuous except at the hilum. (Fig. 16.) The cells are much larger than those of the pericarp. The walls are yellowish brown in color and the lumen becomes filled with a homogenous content. Toward the distal end these cells increase in size, and over the embryo they are thinner and lighter colored.

The micropyle is closed by the cells of the inner integument. The pericarp over the micropyle consists of a distinct inner and outer epidermis with three to four thin-walled cells between. The outer epidermis consists of thick-walled cells with large lumina and the inner epidermis of thick-walled pigmented tubular cells.

Since the seed never becomes detached from its pericarp, there is no true seed scar; however, there is a large opening in the inner integument in the position corresponding to the hilum. (Fig. 16.) Stretching over this region is a continuous stratum of several layers of pericarp cells with somewhat thickened walls. It is known as the closing tissue of the hilar orifice and has been described in detail by Harrington (*11*) for the seed of Johnson grass.

The germination of sugar-cane seed has been studied by various investigators, and Krüger (*15*) gives a series of drawings illustrating the stages in the development of the seedling. The material on which Plate 1, B, is based was furnished by B. A. Bourne, Office of Sugar Plants, who grew freshly harvested, hulled, and sterilized seed on agar cultures. He obtained the first indication of germination after 24 hours if he left the Petri dishes containing the seed exposed to the warm rays of the sun.

The primary root grows a few millimeters, develops a few stubby root hairs, and is then displaced by a secondary root. (Fig. 17, E.) This first lateral root as well as those following possess numerous root hairs, and the region of the root cap is usually colored red. After the emergence of the radicle the plumule elongates and the development of the seedling takes its normal course. A median sagittal section of a 36-hour-old seedling is shown in Figure 17, D. The radicle, although already broken through the pericarp, is still inclosed within the coleorhiza. The plumule became bent in the elongation process and was cut obliquely as seen in the picture. The cells of the epithelial layer of the scutellum have somewhat elongated, but there is no indication as yet of starch removal from the endosperm.

SUMMARY

Sugar-cane flowers are arranged in spikelets grouped in pairs, one sessile and the other stalked. Each flower is subtended by two bracts, which form the outer and inner glumes. Inside the outer glume is the sterile lemma inclosing in its turn the fertile palea. The fertile lemma is absent in most of the varieties of *Saccharum officinarum* but is present in the form of a thin, narrow scale in *S. spontaneum* and all its hybrids observed. At the base of the flower, just inside the inner glume, are two short hyaline lodicules. Lastly, the axis bears a whorl of three stamens and an ovary containing an ascending anatropous ovule.

The rachis possesses normally five bundles, two large and three small ones. These bundles split in the region of the rachis joint into a number of traces to supply the floral parts of the sessile flower and the pedicel of the stalked spikelet. One of the large bundles splits into three strands, of which two subsequently fuse again and continue into the next rachis segment, while the third strand extends into the pedicel. The second large bundle forks, one branch extending into the next rachis segment and the other forming the larger part of the vascular system of the sessile flower.

The stalked spikelet of the varieties studied starts development before the sessile one, but the difference gradually lessens so that at the time of fertilization both spikelets are equally far advanced.

Microsporogenesis in the varieties studied is normal. The chromosome number in U. S. 875, which is a Kassoer seedling, is $x=58$; the number in U. S. 1694, which is a P. O. J. 213 seedling, is $x=40$.

The megaspore mother cell is subepidermal in origin. It enlarges without previously cutting off a parietal cell and then divides twice to form the typical tetrad of megaspores, of which the innermost develops into the embryo sac. The latter is separated from the micropyle by several layers of nucellar tissue. Two cells of the outer layer of this tissue are especially large and serve as entrance for the pollen tube.

Both integuments of the ovule are in places more than two cells wide, especially in the region of the micropyle, but the outer integument surrounds the ovule only partly.

The antipodal cells increase in number and become in addition binucleated or multinucleated. Often their nuclei vary considerably in size. At the time of fertilization the antipodal complex degenerates.

The synergids are well developed but remain inconspicuous because of their poor staining reaction. The synergidal caps are well developed, but the nuclei of the synergids are seldom observed.

The polars which always closely adhere to the egg fuse at the time of fertilization, or soon after, and initiate free nuclear endosperm formation, which becomes cellular first in the region of the embryo.

The early development of the embryo does not differ from that in other grasses, the first two divisions of the zygote being transverse. The mature seed also possesses the typical structure of the grass caryopsis.

The seed coat consists of the fused pericarp, of which, however, only the outer and the inner epidermis and the inner layer of the inner integument are recognizable.

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SULPHUR-SPRAY RESIDUES AND THE SWELLING OF TIN CANS PACKED WITH PEACHES[1]

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INTRODUCTION

The varieties of peaches generally grown for supplying the fresh-fruit market are sometimes canned in considerable quantities, especially in years of overproduction. Owing to the nature of these varieties and to the warm summer temperature, the fruit ripens rapidly and becomes soft in a very short time after ripening begins. Notwithstanding prompt picking and handling, many peaches arrive at the canning plant too soft to be peeled with the usual lye-peeling machines. Such fruit is generally canned by being washed and packed in the usual way without peeling. Fruit packed in this way is sold as "pie peaches." Since the adoption of the sulphur sprays for the control of the brown-rot fungus, difficulty has been encountered with this material because of the swelling of the cans in a few months after canning. The extent of this loss varies greatly in different years.

At the request of some of the canners at Fort Valley, Ga., the writers undertook some work upon this problem in 1925 with the hope of gaining a better understanding of these difficulties.

LITERATURE

At the beginning of the work it was apparent that the swelling was due to spray residue, for no trouble had been experienced with lye-peeled material packed in the usual way.

Clough, Shostrom, and Clark (*1*)[2] had already shown that a lime-sulphur spray upon gooseberries caused gas formation when such fruit was packed in tin cans. The gas present was mainly hydrogen, but it was always accompanied by more or less hydrogen sulphide. They also found that the gas formation was considerably retarded when the fruit was canned in a heavy sirup.

Stevenson (*9*) has shown that lime-sulphur spray on cherries causes the swelling of the container when they are put up in plain tin cans. He has also shown that difficulty may be encountered in washing the spray residue from the fruit. He refers to one instance of swelling of cans of unpeeled pie peaches caused by lime-sulphur spray.

EXPERIMENTAL WORK

In 1925 and 1926 lots of fruit that showed the presence of adhering spray material were selected from the orchard. A quantity of fruit that had received no spray whatever was also obtained. The

[1] Received for publication February 1, 1929; issued July, 1929.

[2] Italic numbers in parentheses refer to "Literature cited," p. 40.

unsprayed material was canned without peeling, both with and without exhaust. One portion of the sprayed material was canned without being washed; another was canned after being washed in cold water; one was dipped in hot dilute lye (0.1 per cent was used, and at frequent intervals enough was added to keep the solution slightly alkaline) and then washed by being sprayed with jets of cold water; another portion was canned after being lye peeled in the usual way.

In the unsprayed unpeeled lots and in the lye-peeled lots that had been exhausted for four minutes no appreciable swelling occurred in two years. The unsprayed unpeeled lots and the lye-peeled lots that were not exhausted showed about 10 per cent of "flippers"[3] at the end of two years. In the 1925 pack practically all the unpeeled sprayed material that was given an exhaust—that unwashed and that washed in cold water—swelled 8 to 14 months after canning. In the 1926 pack the lots thus treated required several months longer to develop swelling, and some had not swelled at the end of two years. The unexhausted cans of unpeeled sprayed fruit swelled very much sooner, some in a few weeks after canning, and nearly all swelled in two years. The lots washed in dilute lye and exhausted did not swell in two years. The unexhausted cans of fruit that had been washed in dilute lye contained about 10 per cent of "flippers" or "swells" at the end of two years; that is, they behaved about like the unsprayed unexhausted checks. It is clear that an efficient exhaust will greatly prolong the time required for swelling to occur.

It is very evident from these experiments that spray residue is rather hard to wash from the surface of peaches. This seems to be due to the insoluble nature of the spray residue and to the velvet-like covering of plant hairs upon the fruit. Washing with cold water and even with hot water did not seem to be entirely effective. Immersing the fruit in hot dilute lye for a few seconds and then washing with cold water has proved effective during the two years that this treatment has been used. It has been found possible to dip quite soft fruit into hot dilute lye for 15 to 30 seconds and then to remove the lye by spraying with jets of cold water without causing disintegration.

EFFECT OF VARIOUS FORMS OF SULPHUR

Since it is possible that the sulphur may exist upon the fruit in several forms, it seemed advisable to determine which form is responsible for the swelling of the cans. Accordingly, in 1925 five lots of unsprayed peaches were put up in No. 2 plain tin cans to which were added lime-sulphur spray, free sulphur as flowers of sulphur, hydrogen sulphide, sodium thiosulphate, and sodium sulphite, respectively. The quantity of fruit filled into the cans varied from 325 to 350 gm., and the quantity of water added varied accordingly, the total weight of the contents being approximately 540 gm. The lots of cans were divided into two series; one series received the equivalent of 100 mg. of sulphur per can, and the other received the equivalent of 10 mg. The sulphur was added as the fruit was packed in the cans. After the proper quantity of water had been added, the cans were exhausted and sealed. In the case of the hydrogen sulphide the addition was made subsequent to exhausting and just before sealing.

[3] A "flipper" is a can slightly distended with gas; a "swell" is one distended more firmly or to a greater extent.

The cans containing lime-sulphur spray or hydrogen sulphide showed swelling first, whereas those containing sodium thiosulphate were the last to show swelling. The lots canned as checks contained less than 1 per cent of swells at the end of two years. The lots receiving 100 mg. of sulphur in all the above-mentioned forms swelled within two years; those receiving the equivalent of 10 mg. of sulphur—as hydrogen sulphide, sodium sulphite, or in the form of lime-sulphur spray—showed a few swells at the end of the first year of storage, and at the end of two years 10 to 30 per cent of the cans were swollen; those receiving 10 mg. of sulphur as sodium thiosulphate did not show swelling of the cans in two years.

From these tests it is apparent that sulphur in almost any form will cause swelling of the cans and that sometimes very small quantities will suffice to produce this effect.

In 1926 the experiments were repeated, 100 mg. of sulphur per can being used in the forms employed in the preceding year, and in addition ferrous sulphide and sulphurous and sulphuric acid were included. These were canned both in cans that were exhausted and in unexhausted cans. The unexhausted cans containing sulphur swelled in a very short time, and even the unsprayed checks that were not exhausted developed a few flippers at the end of two years. The properly exhausted cans usually required several months longer to swell. According to MacInnes and Contieri (*5*), hydrogen overvoltage is increased when the gas pressure is decreased. Hence, properly exhausted cans liberate hydrogen with less ease than unexhausted cans. Sodium sulphide, ferrous sulphide, and sulphurous acid caused swelling of the cans, as did the other forms of sulphur. The sulphuric acid sometimes caused swelling, but frequently the cans pinholed before swelling.

It appears from these experiments that it is not hydrogen sulphide alone that causes the swelling. The gas present in the cans is largely hydrogen. The small quantity of hydrogen sulphide present accelerates the liberation of hydrogen caused by the action of the acids of the fruit on the metal of the container.

CORROSIVE ACTION OF SULPHUR ON THE CAN

In examining the lots of cans it was observed that the cans containing sulphur-sprayed fruit or having other forms of sulphur added to the fruit in the can appeared to be much more corroded than the check lots. It appeared that to a considerable extent the gas formation resulted from increased action of the fruit acids on the can.

To throw further light upon the mechanism of swelling and the corrosive action of sulphur upon the can, certain lots of cans were carefully weighed and filled with water or with 0.5 per cent citric acid. Citric acid in this concentration was used because it is the principal acid of the peach and because the total acidity is approximately of a concentration corresponding to 0.5 per cent citric acid. Various forms of sulphur were then added and the cans processed in the usual way, either with exhaust for three minutes or without any exhaust whatsoever. At the end of three months the cans were examined, one end was removed by a smooth cut, the cans were washed, dried, and again weighed. The loss in weight represents the extent of the corrosion that occurred. Much black sulphide

adhered to the cans receiving sulphur. This was removed by adding a little hydrochloric acid to the can for a few seconds and then quickly washing off the acid with water, drying, and weighing. Detailed notes were taken upon the appearance of the container, the odor of the contents, and the presence or absence of gas formation in the can. The results are shown in Table 1.

TABLE 1.—*Corrosive action of various forms of sulphur upon the tin can*

Substance added to the can	Treatment	Condition as to swelling	Odor of H_2S	Black discoloration	Perforation	Loss of weight per can (grams)
H_2O	3 minutes exhaust	Normal	Absent	Absent	Absent	0.011
0.5 per cent citric acid	do	do	do	do	do	.069
	No exhaust	do	do	do	do	.117
0.5 per cent citric acid and 50 mg. sulphur.	3 minutes exhaust	Swelled	Present	Present	do	.525
	No exhaust	do	do	do	do	.662
0.5 per cent citric acid and 100 mg. sulphur.	3 minutes exhaust	do	do	do	do	.679
	No exhaust	do	do	do	do	.651
	Head space filled with nitrogen.	do	do	do	do	.898
	Head space filled with hydrogen.	do	do	do	do	.838
	Head space filled with oxygen.	do	do	do	do	.737
Distilled water and 100 mg. sulphur.	3 minutes exhaust	Normal	Faint	do	do	.156
	No exhaust	do	do	do	do	.176
0.5 per cent citric acid and 100 mg. sulphur as hydrogen sulphide.	do	Swelled	Present	do	60 per cent.	.775
Distilled water and 100 mg. of sulphur as hydrogen sulphide.	do	Normal	Faint	do	Absent	.089
0.5 per cent citric acid and 400 mg. sodium hydrogen sulphite.	3 minutes exhaust	Swelled	do	do	do	1.161
	No exhaust	do	do	do	do	1.095
Distilled water and 400 mg. sodium hydrogen sulphite.	3 minutes exhaust	Normal	Absent	do	do	.237
	No exhaust	do	do	do	do	.270
0.5 per cent citric acid and 300 mg. sodium thiosulphate.	3 minutes exhaust	Swelled	Faint or absent.	do	do	1.201
	No exhaust	do	do	do	do	1.206
0.5 per cent citric acid and 5 c. c. of 5 per cent sulphurous acid.	3 minutes exhaust	do	do	do	20 per cent.	.974
	No exhaust	do	do	do	Absent	1.050
Distilled water and 5 c. c. of 5 per cent sulphurous acid.	3 minutes exhaust	Normal	Absent	do	do	.193
	No exhaust	do	do	do	do	.481
0.5 per cent citric acid and 500 mg. ferrous sulphide.	3 minutes exhaust	Swelled	Present	do	20 per cent.	1.327
	No exhaust	do	do	do	do	1.050
Distilled water and 500 mg. ferrous sulphide.	3 minutes exhaust	Normal	Absent or faint.	Absent	Absent	.020
	No exhaust	do	do	do	do	.025
0.5 per cent citric acid and 500 mg. stannous sulphide.	3 minutes exhaust	20 per cent flippers.	do	Present	do	.363
1 per cent citric acid and 500 mg. stannous sulphide.	do	do	Faint	do	40 per cent.	.674

It is evident from Table 1 that the properly exhausted cans containing citric acid were not greatly corroded but that the unexhausted cans were somewhat more severely attacked. The quantities of sulphur used caused much corrosion, and exhausting seemed to make comparatively little difference in the result. The same is true with cans in which the head space was filled with nitrogen, hydrogen, or oxygen. In most cases where sulphur had been added at least a faint odor of hydrogen sulphide was present. It was always more pronounced in cans containing acid and was faint or absent in cans filled with sulphur and distilled water. Perforations occurred in several lots, and undoubtedly many more would have developed had the experiment been continued longer.

It is noted that no swelling occurred where distilled water and sulphur were canned together, whereas the cans filled with 0.5 per

cent citric acid with sulphur added invariably swelled. It is apparent that the acid of the fruit is the primary cause of the formation of the gas. The sulphur seems merely to accelerate a tendency already present. The cans containing sulphur in such forms that it could combine with the metal of the container, however, were corroded regardless of the presence of the acid. For example, hydrogen sulphide corroded the can whether acid was present or not, but it did not swell the can unless acid was present. Ferrous sulphide in the presence of acid severely corrodes and causes swelling of the can, but when it is canned with water no corrosion and no gas formation occurs.

These experiments indicate that the spray residue reacts upon the metal of the container to form sulphides. If acid is present the metallic sulphides are decomposed and hydrogen sulphide is formed, at least to a small extent. The hydrogen sulphide thus liberated accelerates the liberation of hydrogen gas as a result of the action of the acid upon the metal of the container.

LIBERATION OF GAS FROM IRON AND TIN

To determine whether the formation of the hydrogen gas was the result of the action of the acids upon the tin or the iron of the can, strips of steel plate, such as are used in the manufacture of cans, and plates of block tin of equal size were immersed in citric acid or water in Ehrlenmeyer flasks, with ordinary burettes so attached that any gas formed could easily be detected and measured. In another set of flasks with iron and tin immersed in citric acid the changes in pressure in the flasks were measured by means of manometers. From the changes in pressure the quantity of gas formed could be computed. The two methods agreed in their general result. A number of these tests were set up with the addition of various forms of sulphur, and in no case were appreciable quantities of gas liberated from tin. This is probably due to the high overvoltage of hydrogen on tin, as shown by Newbery (*7*). If oxygen was present the pressure in the flasks was lowered, indicating the absorption of that element. No appreciable quantity of hydrogen was liberated from iron in distilled water, but hydrogen was liberated from iron immersed in 0.5 per cent citric acid whether sulphur was present or not. Liberation of hydrogen was always much more rapid in the presence of sulphur.

The foregoing experiments show that citric acid of the concentration present in the peach will cause hydrogen-gas formation when in contact with iron alone.

ELECTROCHEMICAL NATURE OF THE CORROSIVE PROCESS

Since the iron of the tin can is more or less imperfectly covered with tin, it seems desirable to know why the fruit rarely causes swelling in the absence of sulphur although there is corrosion and gas formation when small quantities of sulphur are present. This seems to find rather adequate explanation in the electrochemical theory of corrosion (*2*, *8*, *11*).

To discover what effect the placing of the metals iron and tin in contact would have upon the nature and extent of corrosion when immersed in peach juice, an experiment was carried out wherein 700 c. c. of juice from the Belle variety was placed in each of four

ordinary fruit jars. In the first jar a piece of iron 3 by 4 inches (7.5 by 10 cm.) such as is used in the manufacture of tin cans was placed; in the second, a piece of block tin of equal size was placed; in the third, a piece of iron and a piece of tin were placed but not in contact; in the fourth, pieces of iron and tin firmly in contact were placed. The jars were then put into a retort and steamed for 20 minutes, after which the clips were snapped down, sealing the jar. By this method most of the oxygen was excluded. Before the pieces of tin and iron were introduced into the jars, the metals were carefully cleaned with hydrochloric acid, dried, and weighed.

At the end of six months the experiments were terminated, and the strips of iron and tin ware washed, dried, and weighed again. In the first jar the iron (alone) lost 0.555 gm.; the tin (alone) in the second jar lost 0.009 gm. In the third jar with the two metals not in contact the iron lost 0.167 gm. and the tin lost 0.164 gm.; in the fourth jar with the metals in contact the iron lost 0.038 gm. and the tin lost 0.212 gm. It is evident that the iron is greatly protected from corrosion by contact with tin, and that tin in contact with iron is subjected to much greater corrosion. These results are in general agreement with the findings of Kohman (*3*) and also with those of Lueck and Blair (*4*). It is noted that the total corrosion occurring in the fourth jar is somewhat less than the total in the third jar and much less than in the first jar, with iron alone. It appears that the iron is protected to some extent when tin is present in the same vessel, even when they are not in contact. These tests would indicate that tin is anodic to iron when they are used as electrodes in a galvanic cell with peach juice as the electrolyte. The tin behaves as the dissolving electrode in such a cell. This would indicate that in ordinary canning operations iron is generally cathodic to tin.

A cell containing peach juice as electrolyte and iron and tin as electrodes was set up. The resulting potential was small, but consistently indicated that iron was cathodic to tin. If there were ferric ions in the electrolyte this result is just what would be expected from their position in the electrochemical series. In accordance with the normal electrode potentials (*10*), divalent iron is above tin and trivalent iron below tin in the electrochemical series. Since oxygen was present to some extent in the cells heretofore described, it seems probable that enough ferric iron was present to give the polarity indicated.

EFFECT OF OXYGEN AND SULPHUR ON POLARITY OF IRON AND TIN

To show the effect of oxygen and sulphur on the polarity of iron and tin, the cells indicated in Table 2 were set up.

The electrode vessels used were wide-mouthed jars fitted with rubber stoppers. These were filled about three-fourths full of citric acid (700 c. c.) as electrolyte. The acid was boiled just previous to being placed in the jars. Through the rubber stoppers a 0.5 per cent citric acid-agar bridge connecting the vessels containing the electrodes was inserted. The electrodes were carefully cleaned with hydrochloric acid, washed with distilled water, wiped with a clean dry towel, and immediately dried for a short time in an oven. They were then inserted through the rubber stoppers. Glass stopcocks were also inserted through the stoppers to pass nitrogen or oxygen

through the electrode vessels as desired. Where the presence of sulphur is indicated, 0.3 gm. of flowers of sulphur was added to the electrode vessel. A little hydrogen sulphide was also added at the time the electrodes were inserted. The cells thus set up were kept in a room with a constant temperature of 30° C.

Diagram indicating the polarity of iron and tin under varying conditions

No.	Diagram of cell				Resulting polarity
1	Iron...	Citric acid, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen excluded.	Tin...	Iron anodic at first, but became cathodic.
2	Iron...	0.5 per cent citric acid, oxygen present.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen excluded.	Tin...	Iron cathodic.
3	Iron...	0.5 per cent citric acid, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, oxygen present.	Tin...	Iron anodic.
4	Iron...	0.5 per cent citric acid, sulphur present, oxygen excluded.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur present, oxygen absent.	Tin...	Iron anodic.
5	Iron...	0.5 per cent citric acid, sulphur present, oxygen absent.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur absent, oxygen absent.	Tin...	Iron anodic.
6	Iron...	0.5 per cent citric acid, sulphur absent, oxygen present.	0.5 per cent citric acid, agar-agar bridge.	0.5 per cent citric acid, sulphur present, oxygen absent.	Tin...	Iron cathodic.

The potential between tin and iron was never very great, and the polarity could frequently be changed by bubbling the gas through one electrode vessel and not through the other. The potential difference was measured at frequent intervals for 20 days. The potential was variable and drifted one way or the other depending upon the rate at which oxygen or nitrogen was bubbled through the vessels. The polarity was quite generally that indicated in Table 2. Since the condition of the surface of the electrodes is so very important, the results obtained here should be considered only as indicating a general tendency. It seems that where oxygen is present the tendency is for iron to be cathodic and where hydrogen sulphide is present the general tendency is for iron to be anodic. This is in general agreement with the normal electrode potentials of tin and of divalent and trivalent iron (*10*). Divalent iron stands above tin, whereas trivalent iron stands below tin in the electrochemical series. The experiments indicate that in ordinary canning operations tin is anodic to iron, but where hydrogen sulphide is present the ferric salts are reduced to the ferrous form, and iron may become anodic to tin. Oxygen also acts to prevent the formation of a polarizing film of hydrogen. The spray residue therefore tends to reverse the polarity between tin and iron and makes the iron the dissolving electrode of a galvanic cell wherever the tin does not completely cover the iron.

The galvanic action between iron and tin does not account for the increased liberation of gas when sulphur is present. When iron is anodic and is being dissolved by an acid the hydrogen must be liberated at the cathode. Since in these experiments tin has never been observed to liberate hydrogen, it would appear that the hydrogen is liberated from the iron directly as a result of localized chemical action upon the iron. When free sulphur was present the liberation of gas from the particles of sulphur has been observed. In this case the electrochemical action was between iron as anode and sulphur as cathode.

The corroding action of an electrolyte is generally slowed down by the accumulation of ions of the corroding metal, and the corrosion process may even be stopped altogether. In this case hydrogen sulphide prevents the accumulation of ferrous and ferric ions and therefore maintains the tendency to dissolve at its maximum value. This must increase the tendency to liberate hydrogen or hydrogen sulphide and also tends to make iron anodic to tin.

POLARITY OF IRON AND TIN AS RELATED TO CORROSION

To determine just how tin and iron react in an electrolyte when sulphur is present, an experiment was performed wherein 700 c. c. of 0.5 per cent citric acid was placed in each of four ordinary fruit jars. The acid was boiled just previous to being placed in the jars. To each jar 0.3 gm. of flowers of sulphur was added. Plates of iron and tin were introduced into these jars as follows: In the first, iron alone; in the second, tin alone; in the third, tin and iron not in contact; and in the fourth, iron and tin placed firmly in contact. The jars were then heated 20 minutes in a steam bath, after which the tops were clamped down and the jars sealed as in ordinary canning operations. They were thus very nearly free from oxygen. After the jars had been stored for 134 days the metal plates were removed, dipped for an instant in hydrochloric acid, then washed with water, wiped with a dry towel, dried for a short time in an oven, and weighed.

In the first jar the iron (alone) lost 1.126 gm.; in the second the tin (alone) lost 0.172 gm. In the third jar, with tin and iron not in contact, the tin lost 0.12 gm. and the iron lost 1.673 gm.; in the fourth, with tin and iron placed firmly in contact, the tin lost 0.013 gm., and the iron lost 2.198 gm.

It is therefore evident that the presence of sulphur causes intense corrosion of the iron. The tin corrodes very much less than the iron. The tin in contact with the iron does not protect it, but on the other hand the tin is protected to some extent from corrosion by contact with iron.

This is the exact reverse of the relationship obtained with the same metals when immersed in peach juice and in citric acid without the addition of sulphur. This seems satisfactorily explained by the relative positions of tin and of divalent and trivalent iron in the electrochemical series. With an oxidizing acid and with more or less oxygen present, as in the peach juice, ferric iron may be present, which would make the tin the dissolving electrode. If hydrogen sulphide is present any ferric iron would be reduced to ferrous iron, and therefore the iron would be the dissolving electrode, or anodic to tin when in contact with it. It seems that where oxygen or an oxi-

dizing acid is present a certain degree of passivity is conferred upon the iron, and the addition of hydrogen sulphide destroys this passivity. The effect of oxygen and oxygen-supplying acids may also partly explain some of the results of Mantell and Lincoln (*6*), of Lueck and Blair (*4*), and of Kohman (*3*). Of course many factors are concerned, such as temperature, nature of the electrolyte, concentration of the metallic ions in the electrolyte, the character of the surfaces of the corroding metals, and their overvoltage values. From these experiments, however, it is clear that sulphur is of considerable importance in canning operations, since many vegetables are known to liberate hydrogen sulphide during sterilization. Even immature fruits may liberate small quantities of hydrogen sulphide when heated. Much more work will be required to determine the exact effect of sulphur with different products and to control its action favorably in the canning process.

SUMMARY

Sulphur-spray residues are sometimes rather difficult to remove from the surface of peaches. The presence of this sulphur in the can causes the formation of hydrogen sulphide and hydrogen gas, with the consequent swelling of the can.

Washing in cold water and even in hot water does not always prove effective in removing the spray material. Dipping the fruit in hot dilute lye for a few seconds and then washing the fruit with jets of cold water has proved effective in the two seasons in which it has been used. It has been found possible in this way to handle rather soft fruit without disintegrating it.

Almost all forms of sulphur in the acid juices of the peach cause the swelling of the can. Lime-sulphur spray, hydrogen sulphide, sodium sulphide, sodium sulphite, sodium thiosulphate, flowers of sulphur, ferrous sulphide, stannous sulphide, and sulphurous acid have caused swelling of tin cans in experimental packs.

Aside from causing the can to swell sulphur has a corroding effect upon it. In addition to itself combining with the metal, it seems to act as a catalytic agent, greatly accelerating the action of the natural fruit acids upon the metal of the container.

In the presence of organic acids most forms of sulphur will cause corrosion and swelling of the can, but in the absence of these no swelling of the can occurs. However, if the sulphur is in such form that it can combine with the metal it will corrode the can in the absence of acid. Thus, flowers of sulphur corrodes the can whether acid is present or not; ferrous sulphide does not cause appreciable corrosion in absence of acid, but in the presence of acid it greatly corrodes the can.

The action of organic acids upon tin does not result in the liberation of hydrogen, but hydrogen is liberated from iron by organic acids. Thus it seems that if the iron in the container could be completely covered with tin the possibility of gas formation would be much less.

The electrochemical theory of corrosion seems to offer a rather adequate explanation of the phenomena here observed.

In ordinary canning the acid juices of the peach act as an electrolyte in which iron is cathodic to tin when they are in contact. If hydrogen sulphide is present this relationship is reversed, and iron becomes anodic and tin cathodic.

In the presence of oxygen or an oxidizing acid, iron is usually cathodic to tin, whereas if a reducing agent such as hydrogen sulphide is present, the iron is anodic to tin when they are in contact. This relationship, together with the fact that hydrogen sulphide accelerates the liberation of hydrogen from iron, seems to account completely for the swelling of the cans in the cases here described.

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STUDIES ON SCLEROTIUM ROLFSII, WITH SPECIAL REFERENCE TO THE METABOLIC INTERCHANGE BETWEEN SOIL INHABITANTS[1]

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INTRODUCTION

During the past decade soil-inhabiting plant pathogenes have received an increasing share of attention from plant pathologists, and a considerable amount of information has been accumulated concerning the importance of various climatic and soil factors, soil temperatures in particular, in the inception and development of disease (*9*, *10*).[2]

While various investigators had previously called attention to the importance of environmental factors in influencing disease expression of various pathogenes, the relative newness of these studies may be judged by the fact that, while such a factor as temperature was substantially recognized as a conditioning agent by Pasteur and his associates in 1878 (*16*) in their study of the immunity and susceptibility of chickens to anthrax, very little attention had been given to such studies, at least by plant pathologists, until the past few years.

It is, therefore, not surprising that a number of phenomena relative to environmental conditions remain to be investigated, and that some results obtained by varying only one or two environmental factors will be materially modified when a number of natural environmental conditions acting simultaneously are carefully studied under controlled conditions. In this article attention will be directed to the interaction of two soil fungi in limiting or enhancing each other's growth. The studies here reported involve principally *Sclerotium rolfsii*, a fungus which seems to be world-wide in its distribution.

Numerous citations may be found in mycological and pathological literature to the interaction of various fungi, bacteria, and protozoa, but as these have been well summarized by Harder (*5*) and more recently by Porter (*18*) there is no need of considering them here. Many of these references concern the inhibitory or stimulatory action of a contaminant obtained in the process of culturing some pathogene. Others refer to the production of fruiting bodies when two strains of some Mucor, Glomerella, or other species of fungi are brought together. Very little is to be found on the influence of inhabitants of the soil in limiting or stimulating growth and parasitism of soil-inhabiting and root-invading plant pathogenes. Porter was concerned largely with the modifications in growth brought about in a large and heterogeneous collection of fungi and bacteria when two or more are brought together. Incidental to this he found that a bacterial species, designated as No. 45, strongly inhibited the growth on culture media of various fungi, including pathogenic species of Helminthosporium and

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[2] Reference is made by number (italic) to "Literature cited," p. 60.

the flax-wilt fungus, *Fusarium lini*. He also found some inhibitory effect on Helminthosporium when seedlings of wheat were grown on soil inoculated with No. 45 as well as with Helminthosporium. On the other hand, he obtained negative results when he attempted to inhibit the pathogenicity of *F. lini* on flax seedlings by using the same bacterium. Porter does not indicate the natural habitat of his No. 45 and notes that it grows so slowly on soil "that it is doubtful whether it would be very useful in checking the normal soil flora."

THE 1928 EPIDEMIC OF CANTALOUPE ROT

One of the reasons which led the writers to use *Sclerotium rolfsii* in these studies was the extraordinary destructiveness of this fungus during the season of 1928. The disease was first noticed about the middle of July in the region around Hope, Hempstead County, Ark., one of the most important cantaloupe centers in the South. It appeared as a soft, mushy rot of both mature and immature fruit. Frequently the rot could be seen on cantaloupes which were not more than one-third grown, and in such cases the fruit failed to mature and quickly rotted. The most noticeable cases, however, appeared on fruits that were reaching maturity. In such instances, when the fruit was about to be severed from the plant, a whitish, moldy growth could be seen on the sides, and as the lower surface was exposed the mold would frequently be found in greater abundance than on the sides, indicating that the portion of the fruit lying next to the soil served as the primary infection center. Very often this region was more or less rotted, the skin being disrupted, and a sweetish, fermented liquid oozing out of the flesh. If close attention were paid to the soil around the rotted fruit the same whitish mold would be seen extending in fan-shaped fashion for some distance from the attacked plant, growing on the soil, during periods of moist weather. In dry weather it would not be noticed on the soil or to any great extent on the fruit.

While the disease here involved is a southern malady of a large number of different plants (28 new hosts (*15*) having been added recently), including potatoes, sweet potatoes, tomatoes, watermelons, cabbages, and beans, it apparently did not cause any serious losses to these crops in 1928.

The same disease was reported in epidemic form in the eastern cantaloupe-growing region, around Norfolk, Va.,[3] about a week after it was identified in Arkansas. There also it appeared to be confined to the cantaloupe fruit and was unnoticed on eggplants in spite of the fact that it had been observed on the latter crop during the seasons of 1926 and 1927. In Arkansas it was found not only around Hope but in other localities where cantaloupes are grown extensively. It thus appears reasonable to suppose that either a particular, specialized strain of the parasite became active in 1928 or that environmental conditions in diverse parts of the country were such as to render the cantaloupe fruit particularly vulnerable. The latter explanation seems to be more tenable, inasmuch as pure-culture isolations from cantaloupes have given the writers successful infections on young tomato plants, with the production of typical stem rot.

[3] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. SCLEROTIUM ROT OF CANTALOUPE. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 12: 64-65. 1928. [Mimeographed.]

While the southern rot has for many years been known as a storage as well as a field disease, it has rarely been found to act exclusively as a disease of the fruit. In the field it usually attacks the young stems, close to the soil line, causing primarily a rot of the cortex although it may also involve the woody cylinder. Sometimes it has been found attacking older stems and producing similar symptoms. Evidently conditions governing stem infections precluded such attacks in 1928. The nature of these limiting conditions remains unknown. There is, of course, the possibility that some stem rot did occur but went unnoticed or was attributed to some other cause.

FIGURE 1.—Cantaloupes that had been in transit for 24 hours attacked by *Sclerotium rolfsii*. Note the severe rot induced by this fungus on A and the mycelial development on the newspaper wrappers around B

Another factor which makes the recent epidemic of special interest is that the malady would often go unnoticed on the fruit when it was first crated, but when it arrived at the terminal markets the inspector reported considerable "Sclerotium rot." On fruit in transit the fungus has been known to grow with such rapidity as more or less to cover the wrapping papers that happened to be in contact with the fruit. Within 24 hours after shipment it has been found spreading over sheets of newspaper that had been wrapped around the cantaloupes. (Fig. 1, B.) This fungus can very readily utilize cellulose for nutrient purposes, as was determined by growing the fungus on moistened, sterilized filter paper. It produced a noticeable, though not heavy, mycelial growth as well as sclerotia on this medium.

From the knowledge now at hand one may hazard a guess as to the reason for the extreme prevalence of this disease in 1928. The disease producer has in the past been known to be widely distributed in the United States over considerable areas south of the Mason-Dixon line, and has at times been reported from more northern regions

(*15*). But in spite of this wide distribution its extreme destructiveness has been more or less limited to certain localities situated in the extreme southern parts of the United States. While it probably was present in the Arkansas cantaloupe fields prior to 1928 it caused insufficient damage to attract attention. In 1927, for example, cantaloupes are not mentioned in the plant-disease survey [4] as hosts for *Sclerotium rolfsii* in Arkansas, although sweet-potato slips and tomato plants are noted as having been attacked. It will be recalled that the summers of 1927 and 1928 were exceptionally moist and rainy over a large part of the country east of the Rocky Mountains, particularly during the earlier part of the southern cantaloupe-growing periods. The United States Weather Bureau reports for 1928 the wettest June on record for Arkansas and also gives positive departures in rainfall during the first half of July for the Hope cantaloupe region. During 1927 with its tremendous rainfall and destructive floods over wide areas, particularly in the Mississippi Valley region, it may be surmised that the parasite had exceptionally favorable conditions for its development. Various investigators (*1*, *17*, *20*, *25*) have in the past reported the marked proclivities of this fungus for moist conditions, and when 1928 in part duplicated these conditions the parasite ran wild.

GROWTH OF SCLEROTIUM ROLFSII ON POTATO-DEXTROSE AGAR VARYING IN H-ION CONCENTRATION

In his studies on *Sclerotium rolfsii* Higgins (*8*) has brought to light some interesting facts concerning the relationship of the growth of this parasite to variations in H-ion concentration. He found this fungus to be markedly inhibited in alkaline media and unaffected or even stimulated in growth in rather strongly acid ranges of H-ion concentration. The inhibitory effect of the former was so marked that it is not surprising to find him recommending in an earlier paper (*7*) the use of lime on infested fields for the control of this fungus and of the disease which it produces. It is quite obvious, however, that a serious objection may be raised to this work, one that Higgins himself was doubtless cognizant of, namely, that he used beef-extract broth for the test medium. This was chosen because it was found to be better adapted for the growth of the fungus than solutions containing mineral nutrients, and because the different levels of pH values could be determined much more readily than on other media by the methods used.

It, therefore, seemed worth while to duplicate Higgins's work, but instead of using materials like beef extract or commercial peptone, substances which are apt to be quite foreign to a soil-inhabiting plant pathogene, it was decided to use a vegetable decoction which in previous tests had promoted excellent growth of the fungus. The medium used was common potato-extract agar to which sufficient dextrose was added to make a 2 per cent solution. It is realized that this is also quite unnatural, but for artificial media a vegetable decoction more nearly approaches certain soil ingredients than do animal products of the type mentioned above. By checking the pH values

[4] McMillan, H. G. Diseases of vegetables and field crops (other than cereals) in the United States in 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 61, 300 p. 1928. [Mimeographed.]

obtained by the ordinary comparator block method against the pH values obtained by using Wherry's double-wedge comparator it was possible to measure the H-ion concentration with an error of less than 0.1 of a pH division. It should be noted that the buffer index of potato extract is relatively high so that the acidity or alkalinity of the color indicators would not be expected in this case to interfere with colorimetric determinations. The turbidity of the medium was not sufficient to prevent accurate comparisons with known standards, and in every instance a tube or capsule was used, depending on the method, containing the unknown without indicator to mask the color of the known standard.

In the first series of tests the basic medium was made according to the following formula: 500 gm. peeled potatoes, 20 gm. dextrose, 20 gm. agar-agar, 1,000 c. c. of distilled water. The cut potatoes were boiled until the pieces were almost ready to fall apart, the fluid filtered several times through closely woven cloth, the other ingredients incorporated, and sufficient water added to make up the desired amount. The medium was divided into 100 c. c. quantities and autoclaved at 15 pounds pressure for about 15 minutes. After sterilization, the required amounts of N/1 NaOH and N/1 HCl were added to each batch so as to give the following series of H-ion concentrations: pH 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, and 9. The acid and alkaline solutions were added aseptically in a small transfer chamber which had been thoroughly steamed. The H-ion concentrations were determined in the manner previously indicated and precautions taken in each case to see that the final pH value of each solution corresponded with the one desired. The tests were conducted in triplicate form, three plates of approximately 10 c. c. of medium being poured for each pH. Finally the Petri dishes were incubated for 24 hours prior to inoculation so as to be sure that contaminations had not occurred.[5]

Higgins (*8*) had found that an equal quantity of inoculum could be obtained with relative ease by using fully developed sclerotia that had been freed from the mycelium. The present writers have determined that while these bodies vary in size, nevertheless their rates of growth in most instances are comparable at a given H-ion concentration on the same nutrient medium. However, it seemed desirable to have a further check on this, so in several instances two sclerotia on one plate were introduced in order to determine the relative quantity of growth that may be expected in a given length of time from different quantities of inoculum. In this manner it was determined that variations in growth at one pH level occur rather frequently even with sclerotia of approximately equal size, particularly during the first 48 hours. But as the period of incubation was increased up to 72 hours the colonies of any one series tended to become equal, and the variations which occurred in no way interfered with the detection of a very marked difference in growth observed at the different levels. All the plates were kept in an incubator maintained at a temperature of about 29° C.

The colonies were carefully measured and notes were taken for a number of days after the inoculations. Forty hours after the introduction of the sclerotia, growth had occurred in all the plates except

[5] The writers are indebted to A. L. Smith for assistance in these initial tests.

one at pH 3, one at pH 7.5, and all at pH 8, 8.5, and 9. At the end of 72 hours all the sclerotia had germinated up to pH 8. From that point upward to pH 9 no growth could be detected. Table 1 shows the amount of growth obtained at the various H-ion concentrations.

TABLE 1.—*Growth of Sclerotium rolfsii at different H-ion concentrations on a potato-dextrose agar medium*

H-ion concentration (pH)	Average diameter in centimeters of colonies at end of 72 hours	Notes taken at end of 120 hours
3	2.3	All plates full of mycelium except one; no sclerotia.
3.5	3.8	All plates full of mycelium; few sclerotia.
4	3.08	All plates full of mycelium; more sclerotia than at pH 3.5.
4.5	3.8	All plates full of mycelium; sclerotia more abundant than at pH 4.
5	3.75	All plates full of mycelium; sclerotia more abundant than at pH 4.5.
5.5	3.3	All plates full of mycelium; same number of sclerotia as at pH 5.
6	3.5	All plates full of mycelium; greatest number of sclerotia.
6.5	3.7	All plates full of mycelium; not as many sclerotia as at pH 6.
7	1.5	Mycelial growth less than at pH 6.5; sclerotia fewer in number.
7.5	1.41	Growth of mycelium more restricted than at pH 7; few sclerotia.
8	No growth	No growth.
8.5	do	Do.
9	do	Do.

Table 1 shows that between the H-ion concentrations of pH 3.5 and 6.5 there are but minor differences in growth of mycelium. The writers believe that these differences are entirely fortuitous and of little significance. There seems to be, however, a noticeable gradient in sclerotial development and if these bodies are to be regarded as part of a vegetative thallus and are not to be compared with true fruiting bodies then it can be said that there is a noticeable difference in vegetative growth between these levels of H-ion concentration. This interpretation would seemingly be all the more proper because of Higgins's (*6*) morphological and cytological studies. He found the mycelium to be binucleate and made up of both fine and coarse threads. Clamp connections in the broad threads were commonly observed. The present writers have noted the same thing. Higgins also noted that the finer types of hyphae enter into the formation of sclerotia, which "first appear as small white tufts of loosely intertwined small branches." He found that the cells within the center of the mass eventually became vacuolate and usually multinucleate The most important of these findings, for the present purposes, are the binucleate mycelium, the clamp connections—these being the characters which with obvious propriety led Higgins to believe that this fungus may be classed with the Basidiomycetes—and the coenocytic nature of some of the cells. These characters as well as the parallel arrangement of hyphae, a phenomenon which the present writers have found to be of common occurrence in this fungus, all suggest the diploid, secondary mycelium of higher Basidiomycetes.

Table 1 shows a noticeable falling off in growth of mycelium, as well as in sclerotial development, when the neutral point, pH 7, is reached. This was particularly evident at the end of 72 hours and less marked as the period of incubation became longer. The explanation of this becomes evident as one notes that beginning with pH 7 and extending into the alkaline range there is a very noticeable

negative departure in growth, ceasing altogether when pH 8 is reached. The indications are clear that given the type and quantity of inoculum contained in a single sclerotium this fungus is markedly inhibited by hydroxyl ions, and that if these are not sufficiently powerful to inhibit growth completely the fungus will in time overcome the initial inhibitory effect by its marked ability to produce acid. The writers have found that within 72 hours after inoculation oxalate crystals could be readily detected in mounts taken from any plate in which growth had occurred. This is in accord with Higgins's results (*8*) in which he found the reaction of alkaline broth to have changed to a distinctly acid one. For example, he notes that broth testing pH 7.5 will after several weeks' growth of the fungus test pH 4 to pH 4.2. Likewise he called attention to the marked ability of this fungus to produce oxalic acid on various media; in fact, he considered the killing of host tissue by this fungus as being due to this acid. The writers therefore feel justified in suggesting that on media of relatively low alkalinity the initial lag in growth will in time disappear because the metabolic products produced by the fungus enables it to overcome the harmful action of the alkaline medium, and this would tend eventually to equalize the growth at various H-ion concentrations. The writers are not suggesting that staling substances produced by the fungus are absent from the media upon which *Sclerotium rolfsii* has been growing, but if they are formed they play little or no rôle in inhibiting growth of this fungus for some time at least after inoculation, for the fungus permeates not only the bottom of a Petri dish but also the sides and top and often protrudes for some distance around the edges, all within the course of about a week. Its exceptional rapidity of growth is to be observed not only in artificial culture but also under natural conditions.

The final and perhaps the most important point shown in Table 1 is the total absence of growth on potato-dextrose agar at pH 8 and above. This is in agreement with Higgins's results on beef-extract broth in which three of the strains utilized failed to grow beyond pH 8.1 and only one strain grew at pH 8.3. It thus appears that comparable results on diverse media may be expected with reference to the limiting action of OH ions on the germination and growth of sclerotia of *Sclerotium rolfsii*. If such limitation could be obtained in soil which is infested with this fungus in the sclerotial stage, then it is quite obvious that a ready available control measure is at hand, and in accordance with this Higgins (*7*) in an earlier paper expressed the belief that the application of lime, 1 ton stone or 1.5 tons hydrated lime to the acre, would prove beneficial. This is not the first time that liming has been suggested as a possible control for this fungus. In 1908 Fulton (*3, p. 7*) found that *S. rolfsii* will not grow on nutrient media unless they have a high degree of acidity.

This suggests that the fungus may find conditions best for its growth only in soils that are more than ordinarily acid. If this is really the case, the use of lime to reduce the acidity of the soil ought to have a good effect in reducing the loss from blight.

As far as the writers know, Nakata (*13*) is the only investigator who has not found this fungus to be adversely influenced by alkalinity. However, as his English summary does not present the details of his work and as his Japanese text in unintelligible to the present writers, it is quite possible that this seeming discrepancy can be explained as

being due to other factors. It will be shown later on that the kind of substratum as well as the quantity and type of inoculum can materially modify the harmful influence of alkalinity. In his later work (*8*), however, Higgins gave up the idea that liming could be relied upon to give satisfactory control because he found it very difficult to maintain a soil reaction above pH 8 throughout a single season. Indeed, even with the use of 5 tons of hydrated lime per acre, he found the soil reaction to be barely above pH 7 and the attacks of the fungus were not inhibited.

A serious objection may be raised to the tests which have just been described, namely, the obvious impropriety of using pure cultures of a soil-inhabiting fungus on media which are free from other microorganisms. Under natural field conditions such relationships do not exist. The fact that plant pathologists as a whole have paid very little attention to the necessity of studying the natural companions of any particular microorganism does not in the least obviate the desirability of such studies. The senior writer has already called attention to this (*23*), and the recent studies of Millard and Taylor (*11*) on the relationship of nonpathogenic strains of Actinomyces in limiting infections of pathogenic strains in the same genus add emphasis to the necessity of such studies.

With this in mind the writers attempted in a preliminary way to determine the response of *Sclerotium rolfsii* to the presence of another soil inhabitant commonly present in the cotton areas of the South, namely, *Fusarium vasinfectum*. (It should be noted that commercial cantaloupe fields in Arkansas are almost entirely within the cotton-growing region of the State.) At the same time it was deemed desirable to check any possible growth-limiting factor, aside from the preponderance of OH ions, which may have prevented the germination of the sclerotia of *S. rolfsii* on those media which tested pH 8 and above. Accordingly those plates which had shown no growth in the alkaline ranges were inoculated with the cotton-wilt pathogene and in every instance growth of this fungus was encompassed. This is in full accord with Neal's work (*14*) on *F. vasinfectum* in which he found this fungus capable of growing on alkaline media testing as high as pH 9. It was thus further ascertained that the concentration of OH ions was probably responsible for the lack of germination of the sclerotia.

One other factor was taken into consideration, namely, the ability of numerous organisms to change the reaction of a substratum during the process of growth. It is now firmly established that an acid medium may change toward the neutral point and, conversely, that an alkaline medium may be rendered neutral or even acid in the presence of a growing organism. In a former article the senior writer (*22*) reported the cotton-wilt pathogene as changing the reaction of a medium from pH 4.2 to pH 6.6. Neal (*14*) obtained similar results and further determined the ability of *Fusarium vasinfectum* to change an alkaline medium toward the neutral point. If these findings are correct, then, unless some other factor intervenes, it should be possible to obtain growth of *Sclerotium rolfsii* on media originally alkaline which, in the process of sustaining growth of some other microorganism, has lost its alkaline properties.

In order to test this theory all plates, originally testing pH 8 to pH 9, which had failed to produce growth of *Sclerotium rolfsii*, and

which had permitted the growth of *Fusarium vasinfectum* were, after 48 hours' incubation of the latter fungus, reinoculated with mycelium of *S. rolfsii*. It should be noted that the original sclerotial inoculum failed to grow and that the cotton-wilt fungus had made considerable growth within the period given. Tests with Clark and Lub's (*2*) indicators clearly showed a change in reaction of the medium around the Fusarium colonies. With the introduction of fresh inoculum abundant growth of *S. rolfsii* took place in every case and it was therefore concluded that the change in reaction of the medium was primarily responsible for the reversal in growth relationship of this fungus. The fact that the initial inoculum in the form of sclerotia had failed to respond to the changed conditions was attributed to the overcrowding of the Fusarium colonies on the sclerotia. It will be shown later that this is not entirely a safe deduction and that the difference in types of medium as well as in quantity of inoculum has a marked influence on growth reactions, irrespective of the OH-ion concentration.

GROWTH OF SCLEROTIUM ROLFSII ON DEHYDRATED POTATO-DEXTROSE AGAR VARYING IN H-ION CONCENTRATION

One of the reasons which originally led the writers to use potato-dextrose agar instead of the beef-broth medium used by Higgins was the difference in growth that one may expect with media of different chemical composition. Thus Rosen (*21, p. 365*) in 1922 showed that at the same H-ion concentration some media may sustain growth while others inhibit it. In view of this fact and because potato-dextrose agar made in the laboratory may be expected to vary, it appeared worth while to check the results previously given by using a more or less standardized medium of similar chemical composition. Use was made of dehydrated potato-dextrose agar manufactured by a well-known commercial concern which specializes in such products. The same type of inoculum was used and the determinations of the various H-ion concentrations were conducted in the same way as those mentioned previously. Table 2 presents in brief the results of these tests, and Figures 2 and 3 show the nature of the growths produced at different H-ion concentrations.

TABLE 2.—*Growth of Sclerotium rolfsii at different H-ion concentrations on dehydrated potato-dextrose agar*

H-ion concentration (pH)	Average diameter in centimeters of colonies at end of 72 hours	Notes taken at end of 120 hours
3	3.6	All plates full of mycelium.
4	4	Do.
5	4.2	Do.
5.5	4	Do.
6	4.6	Do.
6.5	4.7	Do.
6.9	2	One plate full of mycelium, others almost full.
7.5	0.7	Mycelial growth markedly restricted, plates about two-thirds filled.
8	Faint growth	Average diameter of colonies, 2 cm.
8.2	No growth	Average diameter of colonies, 1.5 cm.
8.5	do	Average diameter of colonies, 1.2 cm.
9	do	Slight growth in all plates.

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An inspection of Table 2 reveals the marked superiority of the dehydrated medium over the ordinary potato-dextrose agar in promoting the growth of this fungus in practically the whole range of H-ion concentration. Although the same general sort of relationship to acidity and alkalinity can be observed on this medium (figs. 2 and 3) as in the one previously given, it will be seen that at pH 8 and above, unlike the former, growth at the end of 120 hours is not entirely inhibited. Why this difference should exist in media of

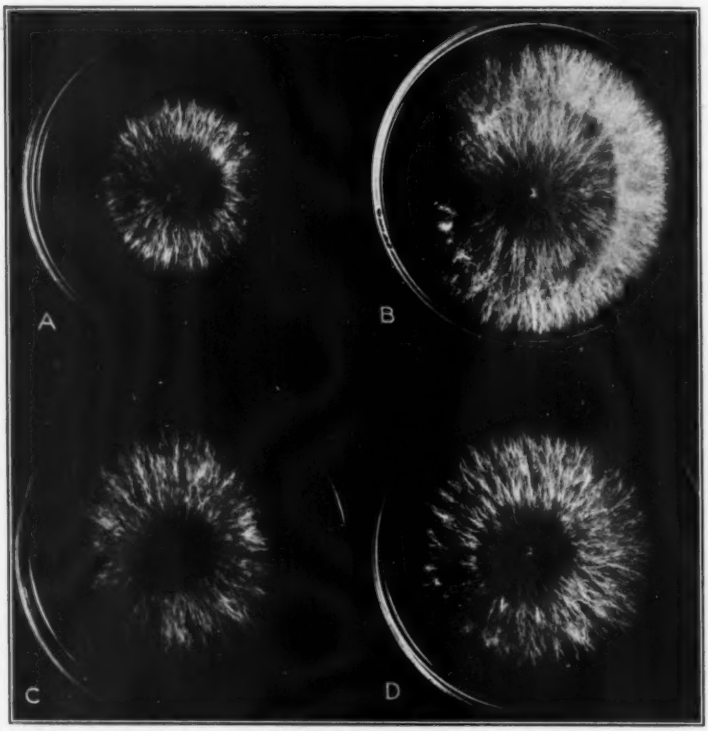

FIGURE 2.—Ninety-six-hour growth of *Sclerotium rolfsii* on dehydrated potato-dextrose agar of different H-ion concentrations: A, At pH 4; B, at pH 5; C, at pH 5.5; D, at pH 6. The original inoculum in the form of a sclerotium is still visible in the center of each colony; compare with Figure 3

similar composition is not entirely clear, but there can be little doubt that the dehydrating process has changed the substance to such an extent as to suggest a marked difference in the amount and kind of nutrients as well as in buffer capacity. The buffer index was undoubtedly different in the two media. The writers' notes show, for example, that with the same initial H-ion concentration it took 0.2 c. c. and N 1 HCl to bring 100 c. c. of the ordinary potato-dextrose agar to a H-ion concentration of pH 5 when 0.35 c. c. of N 1 HCl was required to bring 100 c. c. of the dehydrated medium to the same end point. It would thus appear that the dehydrated material was

much more highly buffered, and this may in part explain the difference in growth. It may be concluded then from these tests that the OH-ion content by itself is not as highly specific in inhibiting growth as was indicated in the former experiments and that the buffer capacity, involving in this case the freedom of activity of the OH ions, has considerable influence on growth response.

FIGURE 3.—Ninety-six-hour growth of *Sclerotium rolfsii* on dehydrated potato-dextrose agar at different H-ion concentrations: A, At pH 6.5; B, at pH 6.9; C, at pH 7.5; D, at pH 8.5. Note the marked decrease in growth around the neutral point and beyond that into the alkaline range, with complete inhibition at pH 8.5; compare with Figure 2

METABOLIC INTERCHANGE BETWEEN SCLEROTIUM ROLFSII AND FUSARIUM VASINFECTUM

The possible influence of *Fusarium vasinfectum* in stimulating the growth of *Sclerotium rolfsii* on alkaline media has already been mentioned. It seemed worth while to investigate this problem more thoroughly. Three series of plates were run simultaneously, dehydrated potato-dextrose agar being used. The first series, which has just been described, was designed to determine the growth relationship of *S. rolfsii* to H-ion concentration when no other microorganism was present; the second was to determine the same thing for another soil inhabitant, *Fusarium vasinfectum;* and the third consisted in

bringing together the two fungi at different levels of H-ion concentration in order to determine any possible interaction between the two. Altogether the three series consisted of 125 plates involving in most instances a triplication in any one test.

The medium was well adapted to promote growth of *Fusarium vasinfectum*, the colonies when grown alone developing well, with of course some differences, in practically the whole range of H-ion concentrations under investigation, from pH 3 to pH 9. But when

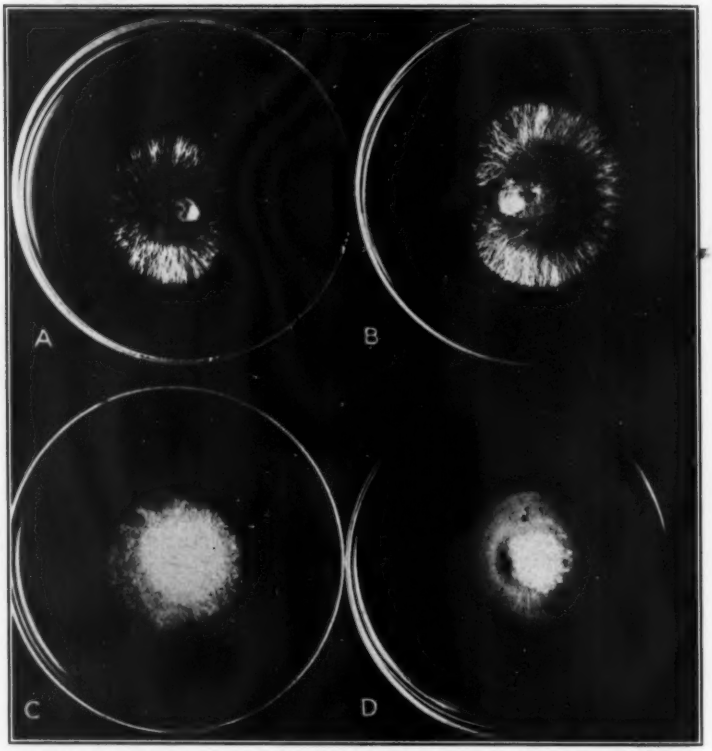

FIGURE 4.—Ninety-six-hour growth of mixed cultures of *Sclerotium rolfsii* (coarse mycelium) and *Fusarium vasinfectum* (fine, compact mycelium) on dehydrated potato-dextrose agar at different H-ion concentrations: A, At pH 4; B, at pH 5.5; C, at pH 6.9; D, at pH 8.2. Note the preponderance of *S. rolfsii* on the acid media and the overwhelming growth of *F. vasinfectum* around the neutral point and on the alkaline side; at pH 6.9 the sclerotial inoculum is so overrun by the Fusarium as to be hardly observable

inocula of the two fungi were introduced into the same plate at approximately the same time and placed near each other, markedly different effects were observed. In the acid media below pH 6.9, *S. rolfsii* completely outgrew the Fusarium, so that within the space of a 4-day interval the latter, while still noticeable as a small pinkish, compact colony, was almost completely surrounded by the whitish, coarse growth of the Sclerotium. (Fig. 4.) As the period of incubation was increased so much of the Fusarium on the acid media was

covered by the Sclerotium that, had it not been for the marked color production of this strain, it would have been entirely lost sight of. (Fig. 5, C.) Whether Fusarium was killed by this overgrowth was not determined.

The reverse phenomenon occurred near the neutral point and in the alkaline ranges. At pH 6.9 and above the sclerotia failed at first to germinate and within four days the fusarial colonies had found their

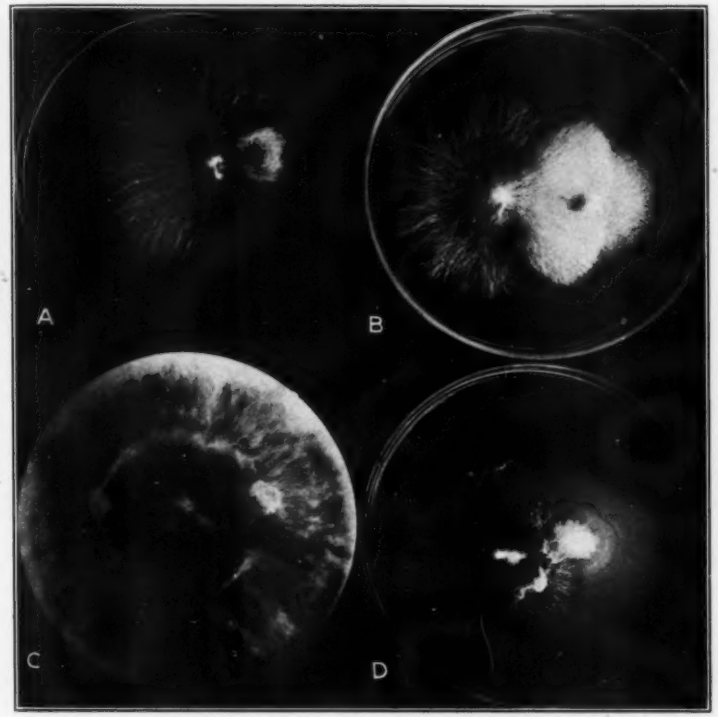

FIGURE 5.—The interaction of soil inhabitants when introduced on a potato-dextrose agar medium which had previously sustained growth of one of the fungi. Following a 4-day growth of *Fusarium vasinfectum*, observable at the right of each plate, there is a 2-day growth of *Sclerotium rolfsii* on A, B, and D, the media having originally H-ion concentrations as follows: A, pH 3; B, pH 5; D, pH 9. C is the same as B of Figure 4, or pH 5.5, photographed 48 hours later and showing the complete overrunning of the Fusarium by Sclerotium, on acid media when the two are grown simultaneously. Note the overrunning of the Fusarium colonies in A and B and the shunning of the medium immediately surrounding the Fusarium by Sclerotium. Contrast this with D, where, in an alkaline medium, Sclerotium is growing immediately around the Fusarium colony as well as upon it

way under the sclerotia or had grown over them so completely as to hide them from sight. (Fig. 4.) Eventually a few of the sclerotia sent out some feeble hyphae, but these were never able to compete successfully with those of the Fusarium. It is of course conceivable that if the Petri dishes had been of greater depth and had contained more media, which would not have dried out as rapidly as in the ordinary plates, the sclerotia might in time have overcome the initial retardation in growth, but if one attempts to envision the

possible behavior of the two fungi under natural field conditions, given H-ion concentrations similar to those under discussion, it is difficult to see how under the ordinary weather conditions prevalent in the South the sclerotia would be more favored than in these artificial cultures. On the other hand, with exceptionally moist periods, it is entirely possible that it might be.

Summing up the results obtained in the interaction of the two fungi when inoculated simultaneously, it may be noted that in the acid ranges *Fusarium vasinfectum* is markedly suppressed while in the alkaline ranges *Sclerotuim rolfsii* has suffered. Comparing this with the growth responses of the two when grown separately, it is seen that the effect is mostly on the Fusarium, while Sclerotium has not been greatly influenced.

The next question is what influence will these fungi have on each other when one is introduced some time after the other has had an opportunity to make growth. To determine this point the following experiment was made. Colonies of *Fusarium vasinfectum* were grown for four days in triplicate on dehydrated potato-dextrose agar possessing the reactions pH 7.5, 8.2, 8.5, and 9. At the end of 96 hours, when fair-sized colonies of this fungus had developed, a single sclerotium of *S. rolfsii* was introduced into each plate close to the margin of the Fusarium colony. After an incubation of 48 hours the sclerotium in each instance was found to have germinated. In most cases the Fusarium colony had continued its growth and had even pushed a flat wedge of hyphae beneath the sclerotium, but this in no way interfered with the germination of the sclerotia and with the subsequent development of the colony. It thus appears that the changes in the medium induced by Fusarium permitted the germination and growth of sclerotia in a time interval which is entirely inadequate to permit growth when the sclerotia are used alone. An inspection of Table 2 shows that no growth occurred on media testing pH 8.2, 8.5, and 9 after 72 hours of incubation. A study of other plates of this series which had also sustained growth of Fusarium for four days was made to determine any possible change in reaction of the medium, and in every instance it was found that the reaction of the medium adjoining the colony had changed toward the acid side of the H-ion concentration. There may have been other substances which stimulated the sclerotia of *S. rolfsii* into growth, but in the absence of any knowledge of such stimulatory substances and in view of the fact that Fusaria instead of producing such materials are known to give rise to staling products that are inhibitory to growth (*19*), it seems reasonable to conclude that the change in reaction of the medium was primarily responsible for the immediate growth of the sclerotia.

MORPHOLOGICAL RESPONSES OF SCLEROTIUM ROLFSII IN MIXED CULTURES

Up to the present we have been concerned mostly with the rates of growth of *Sclerotium rolfsii* when grown alone or in the presence of *Fusarium vasinfectum*. Among other influences which remain to be detailed are the peculiar types of growth encountered in *S. rolfsii* when this fungus is brought in contact with Fusarium. One of these is the apparent chemotropic response of *S. rolfsii* to a Fusarium colony in

media of relatively high acidity. Sclerotium in such instances seems to shun the substratum immediately surrounding the Fusarium, and produces a profuse growth on top of the Fusarium. (Fig. 5, B.) In alkaline media this does not occur, the Sclerotium growing indiscriminately on the substratum around the Fusarium as well as upon the latter fungus. These peculiarities were noted when Sclerotium was introduced on plates in which the Fusarium had grown for four days; the chemotropic type of growth was observed particularly at pH 3.0 to pH 5.0, while above this no such effects were detected.

In the light of the previously noted effects on the substratum, the following explanation is suggested for this response. On the strongly acid media the change in reaction toward alkalinity in the vicinity of the Fusarium colony rendered these places unsuitable for immediate growth of the Sclerotium, so that, taking the course of least resistance, the invader grew over the other fungus. It is to be doubted whether the Fusarium mycelium served as nutrient material for the Sclerotium.

Another type of morphological response noted in *Sclerotium rolfsii* when it grew on top of the Fusarium colony was the production of a distinct arborescent type of growth in contrast to the diffuse, fluffy growth which it usually makes on artificial media. (Fig. 6, D, and fig. 7.) There appears to develop a definite orientation of the hyphae into main axes and side branches, and these in turn give rise to sclerotia which develop along the sides and tips of the branches. (Fig. 7, B.) When examined microscopically the hyphae in such cases are seen to run parallel to each other and to be more or less compacted into a single unit. The reason for this response is not clear, but it is quite comparable to the form of growth frequently found in this fungus when it is growing in its natural habitat on soil. This may perhaps be attributed in part to the competition offered by other soil inhabitants in which Sclerotium is able to hold its own by its ability to produce a compact growth.

The arborescent growth may be compared to the secondary mycelium of higher Basidiomycetes. According to Gäumann and Dodge (*4, p. 403*)—

> the differentiation into main axes and branches, the second characteristic of secondary mycelium, is easily noted in artificial cultures. The main axes are well developed; in contrast to hyphae of the uninucleate [primary] mycelium they run almost parallel to each other and lend themselves easily to the formation of rhizomorphs.

Another possible explanation of the production of this arborescent growth is that the fungus is about to produce fruiting bodies and that this growth is analogous to the tertiary mycelium of higher Basidiomycetes. In the latter Gäumann and Dodge (*4, p. 404–405*) state—

> the secondary mycelium does not proceed as such to the formation of basidia but its hyphae intertwine with extensive change of form and often with loss of individuality to form fructifications, tissues, and organs which in their structure and functions are specialized like those of the Cormophyta. All these tissuelike hyphae systems (plectenchyma, etc.) which have grown from the original, uniform, secondary mycelium are called tertiary mycelia, and develop either as rhizomorphs and sclerotia or as fructifications.

Irrespective of the designation of this type of growth as secondary or tertiary mycelium, its occurrence lends appreciable support to the idea that this fungus may be classed with the higher Basidiomycetes. Summing up the data which point to such relationships

we find (1) that the main vegetative body is made up of hyphae possessing clamp connections; (2) that the cells of this body are binucleate, as shown by Higgins (*6*); (3) that this fungus readily utilizes cellulose for nutritive purposes, a feature possessed by many Basidiomycetes; and (4) that it produces under certain conditions an arborescent growth which is differentiated into main axes and side branches.

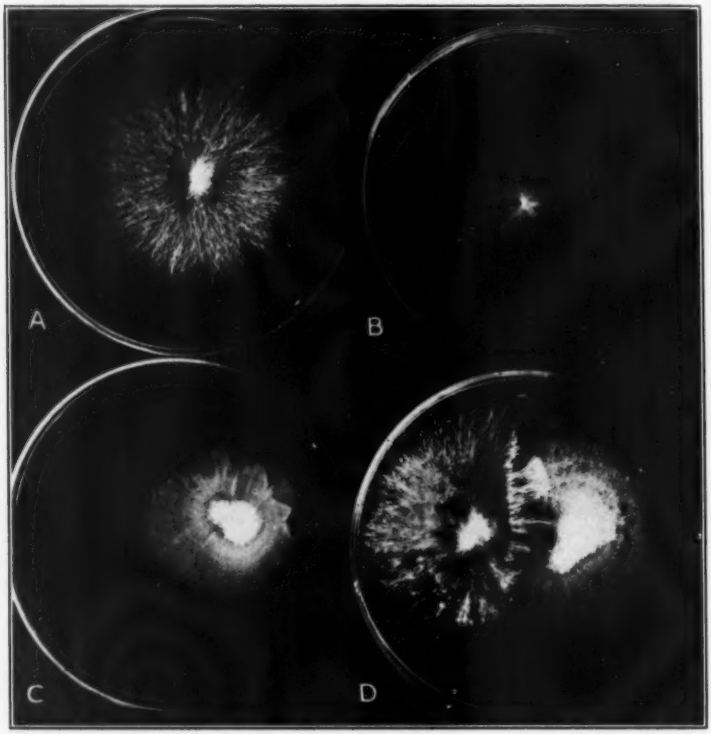

FIGURE 6.—Influence of quantity and type of inoculum in counteracting unfavorable alkaline media; all plates originally tested pH 8.5. A, Forty-eight-hour growth of *Sclerotium rolfsii* when inoculated in form of a relatively large piece of actively growing mycelium; B, 6-day growth of the same fungus when inoculated in form of a sclerotium; C, 4-day growth of *Fusarium vasinfectum* followed by inoculum of *S. rolfsii* in the form of a sclerotium, photographed two days after the introduction of the latter; D, same as C except that the inoculum of *S. rolfsii* was in the form of a large-sized piece of actively growing mycelium. Note the distinct enhancement in growth wherever the inoculum was large and consisted of actively growing mycelium

Using the technic of Stevens (*24*), by which he was able to bring about fruiting in various fungi, including the perfect stages of Ascomycetes, through the action of ultra-violet rays, the writers attempted to induce spore formation in *Sclerotium rolfsii*. Five Petri-dish cultures of this fungus were subjected to ultra-violet rays for approximate time intervals of one-fourth, one-half, 1, 10, and 20 seconds.[6] The plates were held at a distance of about 21 centimeters

[6] Through the kindness of D. M. Moore, of the botany department, University of Arkansas, there was placed at the writers' disposal a quartz mercury arc lamp.

from the light, with one-half of each covered by a black shutter from a photographic plate holder. The colonies were 12 days old at the time of treatment and had completely covered the media. No fruiting bodies were observed on these plates up to 34 days after the ultra-violet application, the media in most instances having almost completely dried out by that time. However, in one plate where drying out had not occurred (the one irradiated for 20 seconds) there was a noticeable fluffy or fuzzy growth covering the sclerotia on the exposed portion of the plate. When examined under the microscope this fluffy material was seen to consist of loosely interwoven and partly free hyphae. But in no case was there any suggestion of basidia formation or of any other spore-forming organs. A careful study of these sclerotia showed that they had failed to mature properly, putting down around the periphery no hardened cells of a brownish color as occurs in normal cases. There appeared also to be a lack of differentiation into a plectenchyma and a central core.

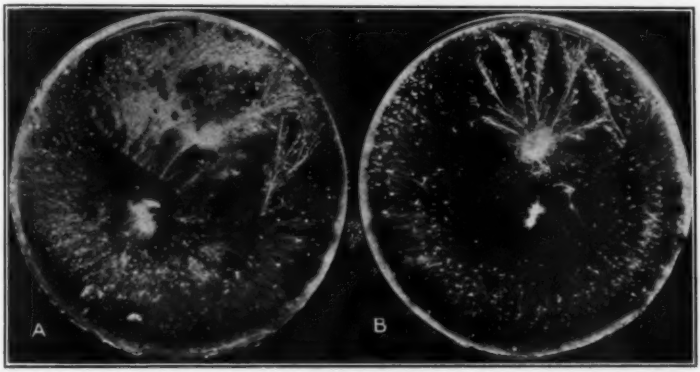

FIGURE 7.—Arborescent type of growth of *Sclerotium rolfsii* when it is growing upon a Fusarium colony. A is the same as D of Figure 6 but photographed six days later. Note how Sclerotium has almost completely overgrown and obliterated from view the underlying Fusarium colonies. The arborescent growth is only visible in those parts of each plate where the Fusarium colony existed

Altogether, the apparent effect produced by the ultra-violet rays on these sclerotia was to keep them in a juvenile condition. These results are offered as suggestive, however, and not as final. There is, of course, the possibility that these bodies, which in gross aspects resemble sclerotia, are either morphological entities capable of giving rise to a spore-forming stage when conditions are favorable or that they represent abnormal growths brought about by the action of the ultra-violet rays.

Quite recently Nakata (*12*), working in Japan, reported that he had found the perfect stage of *Sclerotium rolfsii* to be *Corticium centrifugum* (*Hypochnus centrifugus*). He undertook a series of morphological and cultural studies of 33 strains of *S. rolfsii* and succeeded in obtaining the perfect stage in two strains from Japan and in one from America. A summary of his results, presented in English, indicates that the spore-forming stage is very smiliar to *Corticium solani* (*C. vagum*) and that the main differences are to be found in the mycelium and in sclerotial development.

THE INFLUENCE OF RELATIVELY LARGE QUANTITIES OF INOCULUM OF SCLEROTIUM ROLFSII IN COUNTERACTING UNFAVORABLE ALKALINITIES

The work recorded previously in attempting to determine the growth reactions of *Sclerotium rolfsii* when grown alone or jointly with another fungus was, with the exception already noted, carried out entirely with inoculum consisting of sclerotia. During the course of these investigations there appeared the possibility of inducing growth in this fungus on media inhibitory to the germination of sclerotia but capable of promoting growth of active mycelium. In order to test this out the writers inoculated a series of plates containing approximately equal quantities of dehydrated potato-dextrose agar which had been rendered alkaline by the addition of NaOH. The range in H-ion concentration from pH 8.2 to pH 9, which had previously been found to prevent sclerotial germination for 72 hours (Table 2), was utilized, and the inoculum consisted of relatively good-sized pieces of actively growing mycelium. These transplants were cut out in the form of about 8-mm. squares from tubes containing the same medium, the squares of inoculum consisting of underlying substratum as well as mycelium. Within 24 hours a vigorously growing colony had developed in the entire reaction range, and within 48 hours the growths in most cases covered more than half of the available growing area. (Fig. 6, A.) With the exception of the extreme range of pH 9, no marked difference in growth rates could be detected, and even at pH 9 the growth at the end of 48 hours was fully as good as that obtained in most of the acid ranges in the same time interval when a single sclerotium was used as inoculum. It may therefore be concluded that the type and quantity of inoculum are very important factors in initiating growth and, given the proper substrata and a suitable inoculum, growth may be expected to occur irrespective of the reaction.

SUMMARY

There exists very little information at present on the influence of other microorganisms present in the soil on the growth and activities of soil-inhabiting plant pathogenes.

The very severe epidemic of Sclerotium cantaloupe rot in Arkansas in 1928 suggested the desirability of utilizing *Sclerotium rolfsii* as one of the fungi to be studied. The outstanding features of this epidemic are discussed and evidence is presented to show that while cantaloupe fruit was the only plant part found infected in the field, the strains isolated from these fruits could readily parasitize other plants and other plant parts.

It is shown that *Sclerotium rolfsii* can easily utilize cellulose for nutrient purposes.

Studies on the growth of *Sclerotium rolfsii* on potato-dextrose agar at various H-ion concentrations reveal the fact that the fungus on this medium is markedly tolerant of acid conditions and is quite sensitive to alkaline reactions. There is also a noticeable gradient in sclerotial development on acid media. Beginning with pH 8 and extending upward, sclerotia failed to germinate on this medium.

Fusarium vasinfectum, the other soil inhabitant here studied, grew well on this medium throughout the range of H-ion concentration under investigation, that is, from pH 3 to pH 9.

Since *Fusarium vasinfectum* is known to change the reaction of a substratum upon which it is growing, turning a strongly acid medium toward alkalinity and an alkaline medium toward acidity, this fungus was grown on potato-dextrose agar having those alkaline reactions of pH 8 to pH 9 which had inhibited growth of sclerotia. When reinoculated with *S. rolfsii* these Petri-dish cultures showed abundant growth of the latter fungus.

In order to determine the responses of these microorganisms to a standardized medium of similar composition and to check the results of the previous experiments, use was made of a commercial dehydrated potato-dextrose agar. *Sclerotium rolfsii*, in quantities of inoculum similar to those used in the previous tests, made a greater growth on this medium than on ordinary potato-dextrose agar throughout the range of H-ion concentration investigated, from pH 3 to pH 9. For the first three days the sclerotia failed to germinate on the media testing pH 8.2 to pH 9, but eventually they made growth on these.

The dehydrated product was found to have a much higher buffer capacity than the homemade potato-dextrose agar, and it is concluded that the hydroxyl-ion content by itself is not as highly specific in inhibiting growth of *Sclerotium rolfsii* as was indicated in the former experiments.

Dehydrated potato-dextrose agar was also found to be well adapted to promote growth of the cotton-wilt fungus, *Fusarium vasinfectum*.

When the two fungi were grown simultaneously on the same plate containing the dehydrated medium, different effects were obtained. In the acid media below pH 6.9 *Sclerotium rolfsii* completely outgrew and in time entirely covered the Fusarium, while around the neutral point and in the alkaline ranges the reverse phenomenon occurred.

With the introduction of *Sclerotium rolfsii* into plates that had previously sustained growth of *Fusarium vasinfectum*, still other results were obtained. In such cases dehydrated media, originally rendered alkaline, permitted the germination of sclerotia in time intervals that are unsuitable for germination when *Sclerotium rolfsii* is grown by itself.

Negative chemotropic responses were noted in *Sclerotium rolfsii* on media originally possessing acid reactions which had, prior to the introduction of this fungus, been used for growing *Fusarium vasinfectum*. This response resulted in the overgrowing of Fusarium when the surrounding medium remained uninhabited.

The growing of *Sclerotium rolfsii* on top of a Fusarium colony resulted in an arborescent growth of the former consisting of the production of main axes and side branches. These in turn were found to be made up mostly of parallel running hyphae.

Arborescent types of growth are noted to be common when *Sclerotium rolfsii* grows naturally on soil. Attention is directed to the fact that this type of growth may offer a valuable clue to the relatives of this fungus, and several points are enumerated which indicate its possible relationship to the higher Basidiomycetes.

When attempts were made to induce spore formation in *Sclerotium rolfsii* by means of ultra-violet rays, negative results were obtained.

It was found that the type and quantity of inoculum of *Sclerotium rolfsii* are very important factors in overcoming reactions that are unfavorable to the germination and growth of sclerotia.

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THE RATE OF SPREAD OF POTATO VIRUS DISEASES IN WESTERN NEBRASKA[1]

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INTRODUCTION

The rate and distance of the spread of potato virus diseases has been the subject of considerable speculation and some experimentation because of its great importance in the production of disease-free seed potatoes. Various investigations have shown that both rate and distance of spread will vary in different localities and in successive seasons in the same locality. It is therefore impossible to forecast the amount of spread in one locality from the results of investigations conducted under different environmental conditions. The only experiments dealing with the subject which have been conducted under western dry-land or irrigated culture are those reported by Werner[3] in a study of the spindle-tuber disease.

Spindle tuber has for some time been recognized as the most prevalent virus disease in western Nebraska; the various mosaics are not so common; and leaf roll seldom occurs. This order of relative importance is the reverse of that observed in the eastern part of the United States.[4] It was therefore thought probable that the factors involved in the spread of these diseases in this western area were probably very different from those occurring in the East.

The present investigation was outlined to determine if possible the reasons for the above facts and to gain information which might be of value in the control of these diseases. Repeated observations have shown that these diseases often spread more rapidly under irrigated than under dry-land conditions, and therefore the experiment was designed to include both types of culture. Unfortunately the dry-land plot had to be discontinued after the second year, but the results obtained from studying the relative spread of four potato virus diseases—leaf roll, mild mosaic, rugose mosaic, and spindle tuber—under irrigation are presented for the four years 1924 to 1927, inclusive.

METHODS OF INVESTIGATION

Two different methods of experimentation have been employed in previous investigations of this type: (1) The sampling of commercial fields at different distances from the source of infection, as used by Folsom[5] et al., and (2) the replanting hill by hill, in the same relative position, for several successive generations, of plants which have

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[2] The author is indebted to R. W. Samson for the survey and identification of insects in 1925 and 1926, and to Karl Koch for similar assistance in 1927. Published with the approval of the director as paper No. 72, Journal Series, Nebraska Agricultural Experiment Station.

[3] WERNER, H. O. THE SPINDLE-TUBER DISEASE AS A FACTOR IN SEED POTATO PRODUCTION. Nebr. Agr. Expt. Sta. Research Bul. 32, 128 p., illus. 1926.

[4] FOLSOM, D., SCHULTZ, E. S., and BONDE, R. POTATO DEGENERATION DISEASES: NATURAL SPREAD AND EFFECT UPON YIELD. Me. Agr. Expt. Sta. Bul. 331, 112 p. 1926.

[5] FOLSOM, D., SCHULTZ, E. S., and BONDE, R. Op. cit.

been exposed to infection, as employed by Murphy [6] in the study of leaf roll. The chief advantage of the first method lies in the fact that the diseases are being studied under practical field conditions. This advantage is sacrificed in the second method for the greater accuracy made possible by the more detailed study under conditions having fewer variable and uncontrolled factors. The latter method was used in this investigation.

The investigation was carried out at the Scotts Bluff substation, Mitchell, Nebr. The irrigated plot was isolated in a cornfield each year and was at least 100 to 300 feet distant from the nearest potato field. It was impossible to arrange the plot so that the direction of spread could be studied for successive years. The dry-land plot was located on the same farm but was on a lighter soil to which no irrigation water was applied. It was bordered by fields of corn, wheat, and alfalfa.

The plots used were small in size and consisted of 12 rows spaced 3 feet apart, with 27 hills to the row spaced 18 inches apart. Healthy Bliss Triumph tubers which had been indexed in the greenhouse were used as the source of seed. The term "greenhouse indexing" as used in this paper refers to the removal of one seed piece from a potato. This seed piece was planted in the greenhouse during the winter under conditions favorable for the development of the symptoms of the disease being studied. The remainder of the tuber was planted in the field the following spring. Seed pieces from each tuber were planted in both the dry land and irrigated plots.

Seed tubers obtained from plants affected with the virus diseases to be studied were planted in each plot as follows: Four leaf-roll plants were grown in row 4 as hills 4, 5, 6, and 7 and four mild-mosaic plants as hills 21, 22, 23, and 24. In the same way four rugose-mosaic plants were grown in row 9 as hills 4, 5, 6, and 7 and four spindle-tuber plants as hills 21, 22, 23, and 24. Thus the sources of infection for these four virus diseases, which will hereafter be referred to as the original disease units, were located, one in each corner of the plot. These original disease units are represented in Figures 1 and 2 by the dotted circles.

At the time this experiment was started the distinguishing symptoms of spindle-tuber and the very similar disease unmottled curly dwarf had not been clearly defined in the Bliss Triumph variety. As a result the original spindle-tuber units may have included some unmottled curly dwarf which had simply been diagnosed as severe spindle tuber. In this paper the term "spindle tuber" is therefore used to include both diseases.

One of the usual difficulties in an experiment of this type is the partial or incomplete infection of a hill. To eliminate this trouble as far as possible, each hill was cut back to a single stalk every year. Notes on the symptoms appearing in the field were made at one to two week intervals. Each hill was harvested separately, and a record was made of all symptoms of spindle tuber appearing in the tubers. One tuber from each hill was then selected for indexing in the greenhouse. In some instances a hill had to be classified as questionable spindle tuber, in which case the tuber exhibiting in the

[6] MURPHY, P. A. INVESTIGATION OF POTATO DISEASES. Canada Expt. Farms, Div. Bot. Bul. (2) 44 86 p., illus., 1921.

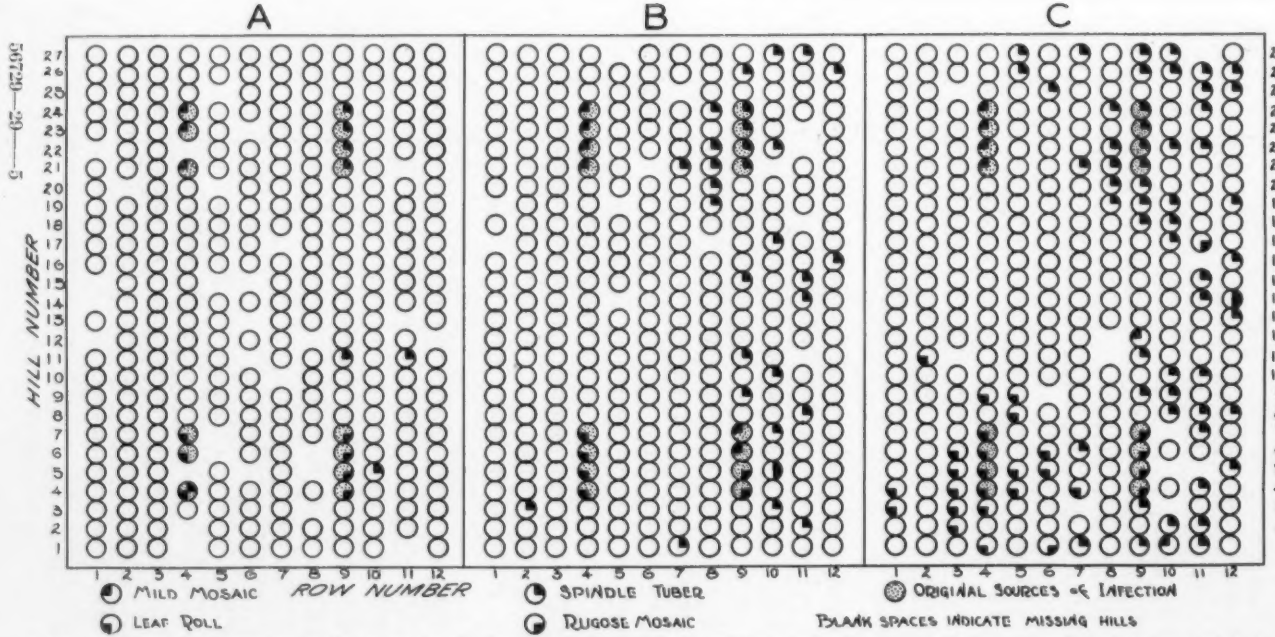

FIGURE 1.—Diagrammatic representation of the irrigated transmission plot for the years 1924 (A), 1925 (B), and 1926 (C). Each circle represents a hill planted in 1924 with healthy seed, except as noted by symbols. Each hill was cut back to a single stalk and one seed piece from each was planted in the same position the following year. The spread of the diseases studied can be followed by attention to the symbols. The plants marked as infected are those having symptoms in the field, but this does not include late-season infection as revealed by indexing (cf. Table 1). Such plants are listed in this figure for the following year when the disease appeared in the field

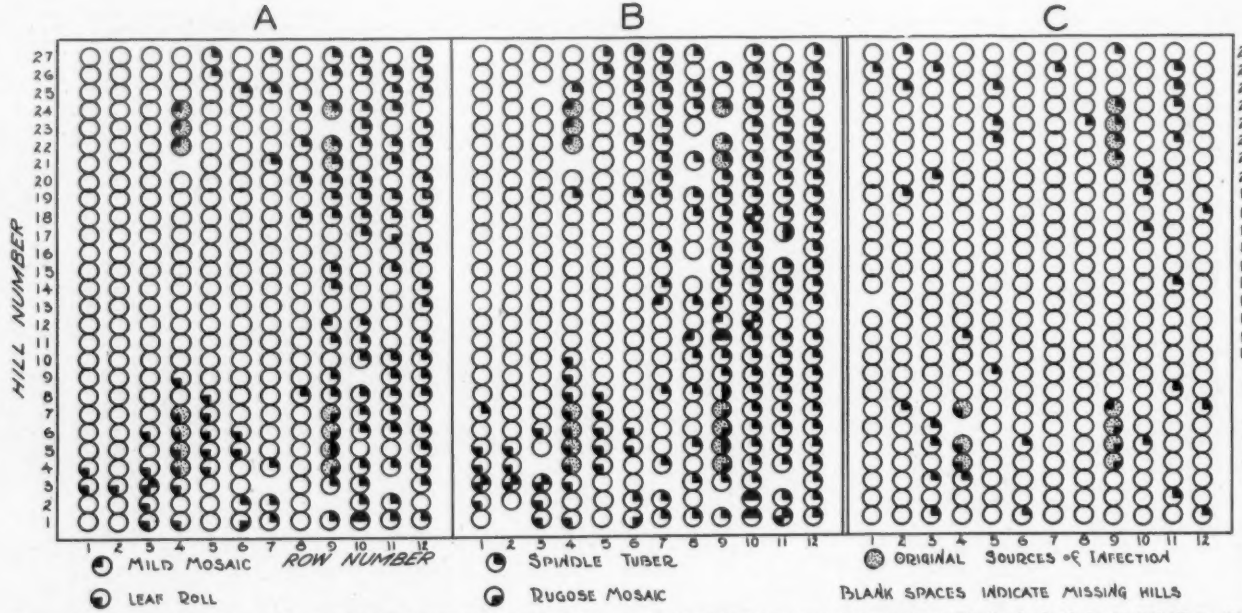

FIGURE 2.—Diagrammatic representation of the irrigated plot for the year 1927 (A); results of indexing the tubers in the greenhouse (B); the dry-land plot for 1925 (C) in which the tubers as original sources of infection in 1924 were planted in a manner similar to that used for the irrigated transmission plot. See Figure 1 for further explanation

greatest degree symptoms resembling those of spindle tuber was selected for indexing. Readings were made in the greenhouse for mosaic, leaf roll, and spindle tuber, and the plants were kept until the tubers had developed sufficiently for the detection of spindle-tuber symptoms. This usually required from two to two and a half months. This greenhouse indexing, which was carried out each year except 1926, was found to be a very great aid in the detection of leaf roll and mosaic, particularly the latter, which, because of the masking of symptoms under high-temperature conditions, is often very difficult to detect in the field in western Nebraska. The indexing in the greenhouse also served to determine the amount of transmission that occurred late in the season of the previous year but did not produce current-season symptoms.

The same tuber indexed in the greenhouse was used for planting in the field the following year. Each seed piece was planted in the plot in the same relative position. In case of missing hills due either to disease or to other causes the hill was planted with healthy seed the following year.

In 1925 and 1927 weekly notes were made of the prevalence of all insect species appearing in the plot. In 1924 and 1926 general observations were made of the insect population at various times throughout the season. No application of either spray or dust was made at any time during the four years.

RESULTS

The progressive spread of the diseases in the plots is shown graphically in Figures 1 and 2. The plants listed as infected are those which exhibited symptoms of the specified disease, either in the greenhouse the previous winter or in the field, and with only one exception the progeny of these hills showed similar symptoms. These plants may therefore have been the result of late infection the previous season or early current-season infection.

In the data presented in Table 1 and in the following discussion, the attempt has been made to determine the time of infection on the basis of the results secured by indexing in the greenhouse. Because of the above facts the figures presented in Table 1 and in the discussion do not check exactly with the data presented in Figures 1 and 2. The inclusion in the diagrams of data on the time of infection would have resulted in a complicated set of symbols and has therefore been omitted.

During 1924 and 1925 no precautions were taken against the spread of these diseases by the cutting knife. In 1924, however, the healthy seed was cut before the infected seed so that no disease could have been transmitted to healthy seed in this way. In 1925 there was no evidence of knife transmission having occurred as all seeds were cut in the same sequence as planted and no infection occurred in the hills immediately following the infected hills of the previous year. This lack of transmission by the cutting knife was probably due to the fact that the seed was cut a considerable time in advance of planting.

By examining Figures 1 and 2 it will be found that many plants listed as infected one year are marked as healthy the following year. In every instance but one this is due to missing hills or to the replant-

ing of infected hills with healthy seed because of an absence of seed tubers. These missing hills are noted in Table 1.

TABLE 1.—*Comparative yearly increase of leaf roll, mild mosaics, rugose mosaic, and spindle tuber in a plot of healthy potatoes grown under irrigation and originally planted with one 4-hill unit of each disease in 1924*

Year	Number of hills [a]	Total of infected hills [b]		New infections		Healthy replants or missing hills of infected seed
		Number	Per cent	Number	Per cent of healthy hills	
LEAF ROLL						
1924	295	3	1.0	0	0.0	1
1925	311	14	4.5	10	3.3	0
1926 [c]	312	26	8.3	12	4.3	0
1927	318	31	9.7	9	3.0	4
MILD MOSAIC						
1924	295	3	1.0	0	.0	1
1925	311	[c] 8	2.6	2	.7	0
1926	312	6	1.9	0	.0	0
1927	313	10	3.2	5	1.6	1
RUGOSE MOSAIC						
1924	295	4	1.3	0	.0	0
1925	311	[c] 6	1.9	4	1.3	0
1926	312	7	2.2	0	.0	1
1927	313	7	2.2	1	.3	0
SPINDLE TUBER						
1924	295	13	4.4	9	3.1	0
1925	311	35	11.3	24	8.0	2
1926 [d]	312	55	14.4	23	9.1	6
1927	313	133	42.4	87	37.3	6

[a] Three hundred and twenty-four seed pieces were planted each year.
[b] The total number of infected hills includes those showing symptoms when indexed.
[c] Two additional mild-mosaic plants were accidentally introduced in place of two rugose-mosaic plants.
[d] No indexing was done in 1926. Therefore part of those listed as 1927 infections may have occurred in 1926. This error does not affect the mild and rugose mosaic readings, as these infections occurred late in 1927.

The following discussion of the spread of these diseases refers entirely to the irrigated plot unless otherwise stated.

LEAF ROLL

The seed in one hill of the original leaf-roll unit planted in 1924 failed to sprout, while a second became infected with spindle tuber, possibly by the cutting knife. These were replaced in 1925 by other seed infected with leaf roll. No spread of leaf roll occurred in 1924. In 1925 no additional leaf-roll plants appeared in the field, but evidently some spread occurred, as 10 new leaf-roll plants were found when the progeny were indexed in the greenhouse. The infection probably occurred late in the summer of 1925. In 1926, 22 leaf-roll plants were found. Of these, 4 were in the original leaf-roll unit while 10 were due to previous season infection as noted above. The remaining 8 plants all showed slight symptoms of leaf roll early in July and were easily identified as leaf-roll plants by the 10th of August.

The disease was evidently caused by early season transmission. In 1927 only 4 new leaf-roll plants were found and these appeared as distinctly diseased early in July. The symptoms were as severe and appeared about as early as in the original leaf-roll unit, thus indicating that infection had probably occurred late in the season of 1926, and they are therefore listed in Table 1 as 1926 infections. The greenhouse index of 1927, however, revealed the fact that 9 new infections had occurred during the summer.

It may therefore be concluded that the greatest spread of leaf roll occurred late in the season of 1925. With three times as many sources of infection present in 1926, only 12 new infections occurred. Eight of these were evidently transmitted early and 4 late in the season. Only a slight amount of transmission occurred in 1927, as evidenced by 9 new infections when there were 22 plants to serve as sources of infection.

It is clearly evident from Figures 1 and 2 that the disease did not spread any great distance. Even after four years all the leaf-roll plants were restricted to that corner of the plot containing the original leaf-roll unit and no new infections appeared at any time more than three or four plants removed from leaf-roll plants. There was no apparent difference in spread from row to row and hill to hill within the row.

MILD MOSAIC

Only three of the four seed pieces in the original mild-mosaic unit sprouted in 1924. No transmission from these plants occurred in 1924 and there were no additional mild-mosaic plants found in the field in 1925. Two of the hills planted in 1925 in the original rugose-mosaic unit in row 9 were found to have been mild rather than rugose mosaic so that in 1925 there were six mild-mosaic plants in the plot. Two transmissions occurred, apparently from these two additional sources of infection, the symptoms appearing in the greenhouse and in the field in 1926. (Fig. 1, C, row 9, hill 12, and row 10, hill 1.) No spread of mild mosaic occurred in 1926 and no additional mild mosaic was observed in the field in 1927. Some transmission, however, probably occurred late in the season of 1927 as the indexed plants in the greenhouse revealed the presence of five new infections. Judging from the location of these hills in the plot (fig. 2, B, rows 7, 8, 9, and 10) none of these infections were transmitted from the original mild-mosaic unit but rather from the two new mild-mosaic plants of 1926, which in turn could be traced to the two mild-mosaic plants accidentally introduced in the original rugose-mosaic unit in 1925.

It is evident that in 1924 and 1926 no transmission of mild mosaic occurred and only a slight amount of spread took place in 1925 and 1927.

RUGOSE MOSAIC

Except in 1925, when two mild-mosaic plants were accidently inserted in place of rugose-mosaic ones, all plants in the original rugose-mosaic unit exhibited the typical symptoms every year. No transmission occurred in 1924 and only one additional rugose-mosaic plant was found in 1925, the symptoms appearing early in September. Three other infections, however, must have occurred late in 1925. as the symptoms appeared on the indexed plants in the greenhouse and in the field in 1926. No new infections were noted in 1926 and

only one in 1927, which probably occurred late in the season as the disease appeared for the first time in the indexed plant. The only instance of partial or incomplete infection of a tuber occurred with hill 14 in row 12 (fig. 1, C), which showed typical symptoms of rugose mosaic in the field in 1926 but failed to show any symptoms of the disease in 1927.

It is clearly evident that only a very slight amount of transmission, five infections, occurred in two of the four years. Two of these were to hills adjoining the original rugose-mosaic unit and the other three were somewhat scattered but none was more than a distance of 12 hills from a rugose-mosaic plant.

SPINDLE TUBER

Although the amount of transmission obtained with the mosaic diseases was slight, spindle tuber spread much more extensively. (Figs. 1 and 2.) The first evidence of spindle-tuber transmission was found when the tubers produced in 1924 were examined. Four plants produced tubers with distinct spindle-tuber symptoms and in addition 5 new infections were found when the tubers were indexed. In 1925 there were 19 new spindle-tuber plants as evidenced by either foliage or tuber symptoms, and 5 additional infections were discovered by indexing. In 1926 there were 26 infections, resulting in the appearance of current-season symptoms on either foliage or tubers. As no index was made in 1926, it is impossible to determine how many of the 38 new spindle-tuber plants that appeared in the field in 1927 were due to transmission late in the season of 1926. The index of the 1927 crop disclosed the fact that in addition to these 38 new spindle-tuber plants there were 49 late-season infections that did not cause symptoms in the tubers but did produce the typical disease when these tubers were indexed.

Even with the large amounts of spindle tuber present after the first year only 42.4 per cent of the plants were infected after four years. Furthermore, the disease did not spread for any great distance after the first year. In 1924, in addition to the original spindle-tuber unit, there were nine scattered infections, four of which are indicated in Figure 1, A. The five additional infections, which were found by indexing and which are listed in Figure 1, B, occurred in hills 1 and 21 in row 7, hill 22 in row 8, hill 9 in row 9, and hill 27 in row 11. Most of the spread in the following years was centered around these plants and the original spindle-tuber unit, with the exception of hill 3 in row 2, which became infected in 1925, and hills 26 and 27 in row 5, infected in 1926. An examination of Figure 2, A and B, might lead one to assume that the spread of this disease occurred chiefly in one direction. As previously noted, however, the plot was not laid out in the same direction each year. It is more probable that the large amount of spread in rows 9 to 12 was due to the distribution of the infections which occurred in 1924 and 1925. (Fig. 1, A, B.) The remarkable thing is that after four years there were only six spindle-tuber plants more than three rows removed from the row containing the original spindle-tuber unit, a fact which showed that the disease did not spread for any great distance. It was also notable that in these four heavily infected rows there still remained a half dozen healthy plants at the end of the four years. These are being tested to determine whether they possess any inherent resistance to the disease.

COMBINATIONS OF DISEASES

It would be expected that in a plot of the type used in these experiments there would be a considerable number of plants infected with more than one disease. It was fairly easy to distinguish disease combinations, with the possible exception of mild mosaic combined with rugose mosaic, and as these two diseases did not spread very extensively it is doubtful that this combination occurred. There were 18 plants infected with spindle tuber in addition to 1 of the other virus diseases, 4 of these were combinations with mild mosaic, 6 were rugose mosaic, and 8 were leaf roll.

RELATION OF INSECTS TO SPREAD

IRRIGATED PLOT

The greatest amount of infection with all four diseases occurred in 1925 and the next largest amount in 1927. Only leaf roll and spindle tuber were transmitted in 1926 and only spindle tuber in 1924.

By checking over the insect survey made on five different dates between July 2 and August 26 in this plot in 1925, it was found that a small number of aphids were present on July 2 and a much larger number on August 26. On the latter date 183 plants were carefully examined and from 1 to 7 aphids were found on each of 38 per cent of the plants. While this could hardly be called a heavy infestation of aphids, it was the largest number found at any time during the four years and probably accounts for the greater amount of transmission that occurred in that year. The other insects noted in 1925 were Colorado potato beetles, gray blister beetles, leaf hoppers, tarnished plant bugs, and grasshoppers. The tarnished plant bugs were the most abundant

In 1927 the only aphids observed were found very late in the season just before digging. This may explain the fact that the only transmission of mosaic and leaf roll occurred late in the season and was not evident until the tubers were indexed in the greenhouse. A considerable number of flea beetles were present early in July and August, but no other insects were noted except Colorado potato beetles, a few leaf hoppers, and some grasshoppers.

In 1924 and 1926 the records of insect infestations were not made as accurately as in the other years, but a careful lookout was kept for aphids both in this plot and in the other experimenal plots on the farm. None, however, were noted.

From these observations it would appear that the few instances of leaf-roll transmision in 1926 and the extensive spindle-tuber transmission in all four years must be explained on some other basis than that of aphid transmission. Grasshoppers,[7] which have been shown to be capable of transmitting spindle tuber, were possibly the chief agents in the spread of this disease, although the other insects present can not be eliminated until further tests of their ability to transmit the virus have been made.

DRY-LAND PLOT

The type of spread of spindle tuber occurring in the dry-land plot was in sharp contrast to that presented above for the irrigated plot.

[7] GOSS, R. W. TRANSMISSION OF POTATO SPINDLE TUBER BY GRASSHOPPERS (LOCUSTIDAE). Phytopathology 18: 445–448. 1928.

The dry-land plot was only used for two years and the detailed data are only presented for one year, 1925. (Fig. 2, C.) This plot was planted in the same manner as the irrigated plot, both the infected and healthy seed being from the other halves of the seed tubers used for the irrigated plot. Because of unfavorable conditions, the stand was very poor in 1924. Only 207 hills were produced from 324 seed pieces planted. Only two plants in addition to the original spindle-tuber unit exhibited the symptoms of this disease and there was no evidence of spread of the other three diseases.

This plot suffered a severe infestation of grasshoppers during 1924 from an adjoining alfalfa plot, the first three rows being almost entirely defoliated. Grasshoppers were also present in 1925. Figure 2, C, shows the amount of transmission which occurred in 1924 as evidenced by symptoms appearing in 1925 in addition to the current-season symptoms that appeared from transmission early in the season of 1925. There were 40 spindle-tuber plants in 1925. Six of these were present in 1924, and 20 of them occurred in plants grown from healthy seed used to replace the missing hills of 1924, so that not more than 14 of these infections could have occurred late in the season of 1924. This total of 16 possible infections in 1924 is much greater than the number that occurred in the irrigated plot, and the distance of spread is greater than in the irrigated plot even after four years. Undoubtedly the grasshoppers were the chief agents in the spread of spindle tuber. In addition to the 20 new infections that appeared in the field in 1925, there were 16 additional instances of transmission as revealed by indexing the tubers. Most of these infections centered around the plants infected in 1924 and were widely scattered over the entire plot.

In 1925 it was determined by greenhouse indexing that there were two instances of leaf-roll transmission and one of rugose mosaic. This transmission was probably due to late-season infection by aphids, which were present in August, though less abundantly than in the irrigated plot. Neither of these diseases was transmitted in 1924. Due to an error the original mild-mosaic unit was not planted in 1925 and one hill of the original rugose-mosaic unit was accidentally planted with mild-mosaic seed. No transmission of mild mosaic occurred in 1924 from the original unit, nor in 1925 from the single plant in row 9.

DISCUSSION

While the experimental plots used in these tests are not strictly comparable to large commercial fields, the results obtained are in harmony with the writer's observations of the spread of these diseases in western Nebraska. The relative amount of spread of these four diseases in any one year and of each disease for the four years portrays quite accurately the conditions existing in the commercial fields of the same area during the same period.

It is clearly evident that mild and rugose mosaic are not readily transmitted under these conditions. The amount of spread was negligible as contrasted with that observed in the eastern part of the United States by other investigators. Leaf roll, while transmitted more extensively, was still limited in extent and the amount of transmission was much less than that reported by investigators in other sections of the country. These three diseases may be chiefly de-

pendent upon aphid transmission, and therefore have not become as serious a menace under western conditions, where aphids are less frequent than in other parts of the country. Murphy,[8] however, reports transmission of leaf roll by capsid bugs (*Calocoris bipunctatus*), jassids (*Typhlocyba ulmi*), and flea beetles (*Psylliodes affinis*). It is therefore quite probable that insects other than aphids were responsible for some of the leaf-roll transmission, particularly in 1926, when aphids were not present.

Spindle tuber was found to be quite extensively transmitted, although not to as great distances as reported by other investigations nor in as large amounts as indicated by Werner.[9] Werner's experiments, conducted in 1923, in which he found a very extensive spread of spindle tuber, are not strictly comparable to the tests reported in this paper, for there may have been in that year a much more severe insect infestation than in any of the following four years. It is clearly evident, however, that this disease is a more serious problem in western Nebraska than the other three diseases studied. The increase from 14 to 42 per cent in 1927 gives some indication of the rapidity with which it may increase when conditions are favorable. It must be remembered, however, that part of this increase probably occurred late in the season of 1926, but was not determined because no indexing was done that year. Judging from the results of these four years' study, which included a considerable range of variation in weather conditions, the disease would hardly be expected to much more than double in amount in an average year. It is also evident that this disease is transmitted by other insects than aphids, and it is quite probable that in addition to grasshoppers it will be found that a number of other insects can act as transmitting agents.

It was found very difficult to detect the mosaic plants in the field because of the masking of symptoms. They were easily distinguished, however, by greenhouse indexing. Leaf roll was usually distinguishable both in the field and by indexing, but under certain field conditions it was very difficult to separate it from severe spindle tuber. The latter disease could usually be detected, however, by the tuber symptoms. Nevertheless, in the final readings for spindle tuber the previous history and the performance of the progeny had to be taken into consideration in order to avoid inaccuracies that would have occurred if the readings for any one year had been considered alone. This difficulty in accurately diagnosing spindle tuber was due, of course, to the number of environmental factors that could seriously affect the intensity of the symptoms.

SUMMARY

Mild and rugose mosaic were transmitted in small amounts in only two of the four years of the experiment, and their spread can be correlated with the occurrence of aphids.

Leaf roll was transmitted slightly more extensively, that is, there was 9.7 per cent infection after four years, but the disease spread only to a distance of about three hills from possible sources of infection. The spread could be correlated with aphid transmission except in 1926, when other insects were probably involved.

[8] MURPHY, P. A. INVESTIGATIONS ON THE LEAF-ROLL AND MOSAIC DISEASES OF THE POTATO. Ireland Dept. Agr. and Tech. Instr., Jour. 23: 20-34. illus., 1923.

[9] WERNER, H. O., Op. cit.

Spindle tuber was transmitted more extensively, that is, there was 42.4 per cent after four years. Transmission occurred in all four years, but was more abundant in 1925 and 1927, when aphids were present. The spread of this disease could also be correlated with the presence of grasshoppers, although other insects may have been involved. None of these diseases were spread more than a few rows distant from the sources of infection.

Of the four diseases studied, spindle tuber is undoubtedly the one most to be feared under western conditions, although there was evidence that leaf roll may become a serious problem if it once becomes established in the West.

THE RESPIRATION FACTOR IN THE DETERIORATION OF FRESH VEGETABLES AT ROOM TEMPERATURE[1]

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Oklahoma Agricultural Experiment Station

INTRODUCTION

The physiological changes that occur during the rest period of the hardy vegetables have been the subject of many investigations. In general, the chemical composition varies slowly, and it has been found convenient to allow rather large intervals of time to elapse between observations. Among the more perishable vegetables, however, Morse,[2] working with asparagus, and Appleman,[3] and Appleman and Arthur,[4] with green sweet corn, have found that profound changes in composition occur within a few hours after harvesting, sweet corn, for instance, losing between 50 and 60 per cent of its sugar during 24 hours at 30° C., and very little after that with longer holding; by using a low storage temperature the loss could be very appreciably reduced. In view of the manner in which fresh vegetables are usually handled in markets, stores, and kitchens it becomes important to investigate the changes occurring at room temperature in other green vegetables during the first few hours after they are gathered, and that was the object of the present study. As respiration is probably the principal factor in the deterioration of fresh vegetables at higher temperatures, attention was focused upon that process.

It is recognized that vegetables in markets and stores are subjected to many variables, all of which probably affect the rate of respiration and the rates of the other processes involved in deterioration; there are external variables such as temperature, humidity, and intensity and kind of illumination, and internal factors involving the variety of the vegetable and its degree of maturity. It would be interesting to determine the influence of each of these variables on the rate of respiration, but that was beyond the scope of this study, and so fixed conditions were chosen—a temperature of 30° C., an atmosphere which was saturated with water vapor, absence of light, and that stage of maturity in each case which represents prime eating condition—and the data obtained from the different vegetables under identical conditions should serve as the basis for comparisons.

EXPERIMENTAL PROCEDURE

A diagram of the apparatus used in the experiments is shown in Figure 1. Soda lime in A served to remove CO_2 from the air that entered from out of doors. The reservoir C and the respiration

[1] Received for publication Feb. 11, 1929; issued July, 1929. Published with the permission of the director of the station.

[2] MORSE, F. W. EXPERIMENTS IN KEEPING ASPARAGUS AFTER CUTTING. Mass. Agr. Expt. Sta. Bul. 172, p. [297]–307. 1917.

[3] APPLEMAN, C. O. RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN. Amer. Jour. Bot. 5: 207–209, illus. 1918.

[4] ——— and ARTHUR, J. M. CARBOHYDRATE METABOLISM IN GREEN SWEET CORN DURING STORAGE AT DIFFERENT TEMPERATURES. Jour. Agr. Research 17: 137–152, illus. 1919.

chamber D were placed beneath the surface of the water in an electrically controlled thermostat. It was assumed that the movement through C would be sufficiently slow to allow the air to assume the temperature of the surrounding bath and become very nearly saturated with water vapor from the layer of water in the bottom of this 2-liter bottle; a dropping funnel (not shown) permitted the addition of more water in C as it was needed. The air was always sufficiently humid to prevent the vegetables from wilting. For the respiration chamber,

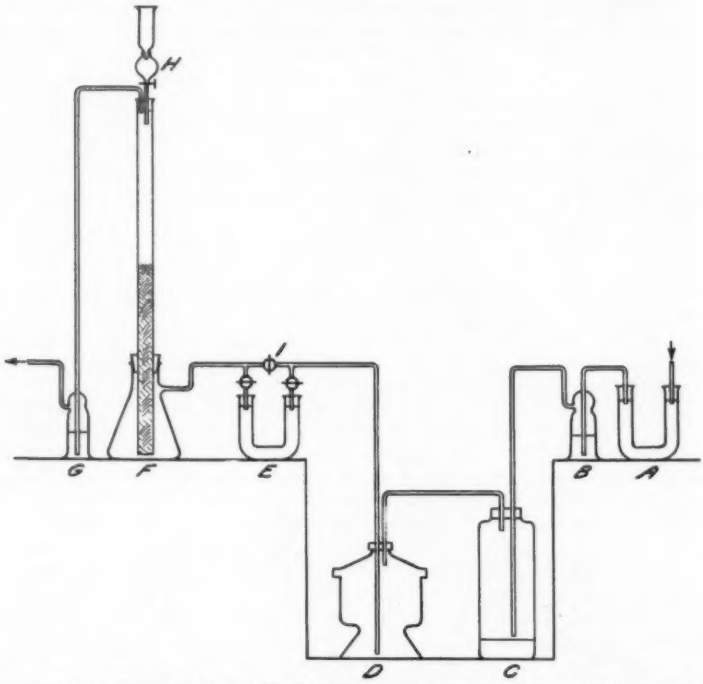

FIGURE 1.—Diagram of the respiration apparatus: A and E contain soda lime; B and G contain a dilute solution of bromthymol blue; C, air reservoir; D, respiration chamber; F, Truog absorption tower; H, 50 c. c. separatory funnel for introducing standard $Ba(OH)_2$ solution; I, stopcock. C and D are placed in the constant temperature bath

vessels of various sizes and shapes were used, the object being to suit the vessel to the vegetable so as to allow as little waste space as possible—wide-mouthed bottles, small desiccators, and sometimes straight tubes 1-inch in diameter, stoppered at each end, served the purpose. The stopcocks at I permitted the gases to be diverted through the soda-lime tube E between measurements. F is the Truog [5] absorption tower with the tube lengthened to 30 inches to lessen the danger of loss of solution at the top. $Ba(OH)_2$ solution was introduced at H. Truog's technic was followed almost exactly in the use of this tower, which proved to be much more efficient than

[5] TRUOG, E. METHODS FOR THE DETERMINATION OF CARBON DIOXIDE AND A NEW FORM OF ABSORPTION TOWER ADAPTED TO THE TITRIMETRIC METHOD. Jour. Indus. and Engin. Chem. 7: 1045–1049, illus. 1915

any other absorption device tested. At B and G small wash bottles were introduced containing a dilute solution of bromthymol blue. The laboratory distilled water had a pH of approximately 8.0 when CO_2 was removed and consequently gave a good deep-blue color with this indicator. The wash bottles, situated as they were, served to show whether CO_2 entered C or escaped from F. On a few determinations with asparagus (the most rapidly respiring vegetable examined) a small white crucible containing a little of the indicator was placed in the respiration chamber D with the vegetable, and gave assurance by its green tint that the rate of movement of the gas stream was sufficiently rapid to prevent the collection of significant amounts of CO_2 in the chamber. In making determinations the water pump was turned on far enough to draw bubbles almost to the top of F, and the indicator bottles were watched to see that they maintained the proper shade; beyond this no attempts were made to measure or control the rate of flow of gases through the train. Sometimes preliminary experiments were necessary to discover the most convenient amount of any vegetable to use; the weights employed varied from 50 to 500 gm. The respiration chamber was submerged beneath approximately a foot of water, in most cases lead plates covered the top to keep it down, and the thermostat was in a corner of the laboratory far removed from windows, so that the influence of light may be considered negligible.

Experimental material consisted of fresh vegetables of edible maturity brought directly from the garden into the laboratory before the sun was high enough to cause any wilting. All unnecessary bruising was avoided. Vegetables were used for study in the forms in which they are displayed on the market; that is, in the case of beet, green onion, carrot, and lettuce, the whole plant was used; in the case of green beans, pimientos, mangoes, and okra, a very short stem was left attached; tomatoes were pulled off carefully from the vine, and asparagus was cut at the surface of the soil. The choice of vegetables was determined by availability. It should be noted that the temperature in the garden when the vegetables were obtained was usually near 30° C., so that the material was not subjected to a decided change in temperature when it was placed in the thermostat. The material was washed, dried carefully, and weighed, only perfect specimens being used.

Thirty degrees centigrade was chosen as the working temperature and the bath was held constant to within $\pm 0.5°$. This particular temperature seemed best adapted to the study for two reasons—(1) it was practically the lowest temperature which could be held constant during the summer months with a water thermostat and no special cooling device, and (2) it represents fairly well the average daytime room temperature in this part of the country (Oklahoma).

Determinations were made as follows: After the material for study had been placed in the respiration chamber D, 25 c. c. of 0.25N $Ba(OH)_2$ was introduced into the funnel H, and the suction of air through the apparatus was begun, the by-pass tube E being used to remove all CO_2 coming from the respiration chamber. When the indicator bottle showed by its color that the CO_2 originally present in the absorption tower had been removed (usually about one-half hour sufficed) the stopcock in H was opened, allowing the $Ba(OH)_2$ solution to enter the tower. The stopcock of H was closed immedi-

ately and the stopcock I opened, at the same time the two stopcocks on the by-pass tube were closed. The exact time of opening I was noted; the runs were continued for exactly one hour, after which I was again closed, the by-pass stopcocks opened, and the suction reduced. The $Ba(OH)_2$ solution remaining on the walls of H was washed into the absorption tower F with small amounts of CO_2=free water. The absorption apparatus was disconnected, the stopper carrying the tube loosened, and the beads washed with CO_2=free water into the flask, where the excess $Ba(OH)_2$ was determined by titrating with N/10 HCl in the presence of phenolphthalein. Determinations were repeated at 2-hour intervals throughout the day. The experimental material was kept in the thermostat overnight, but the lid of the respiration chamber was replaced by a loosely arranged wet cloth and the jar was supported with its mouth on a level with the surface of the water, so as to allow free access of air to the vegetables without allowing them to wilt or change temperature. Determinations were continued then through the second day. Blank determinations showed that no measurable amount of CO_2 leaked in or was taken up directly during the manipulations.

EXPERIMENTAL RESULTS

The results, presented in the form of curves, are shown in Figure 2. In every case where there was a significant decline in the rate of respiration during the first 12 hours the whole 30-hour determination was repeated one or more times with fresh material. The curve given in each case was one chosen as typical. Curves on different specimens of the same kind of vegetable were always closely similar in shape and in actual position. The curves appear to approach the logarithmic in form. By mechanically computing the area under the curves for a 24-hour period (from the second until the twenty-sixth hour) the total amount of CO_2 evolved was determined; by assuming that all the carbon lost came from glucose, the weight of this sugar used up may also be calculated. These values are shown in Table 1.

TABLE 1.—*Calculations of the carbon and glucose lost per 100 gm. dry weight of various vegetables when held at 30 C° for 26 hours after cutting*

Vegetable	Dry matter	Weight of CO_2 evolved between second and twenty-sixth hour	Weight of carbon lost between second and twenty-sixth hour	Calculated weight of glucose lost between second and twenty-sixth hour
	Per cent	*Grams*	*Grams*	*Grams*
Asparagus	8.3	20.066	5.473	13.682
Lettuce	6.2	9.424	2.570	6.425
Green beans	7.7	9.273	2.529	6.322
Okra	13.7	7.699	2.091	5.228
Green onion	9.3	6.627	1.807	4.518
Carrot	13.9	4.659	1.271	3.178
Tomato	6.1	3.920	1.069	2.672
Beet	9.5	* 2.958	.807	2.018
Green mango (sweet pepper)	8.0	2.880	.758	1.962
Red pimiento (short, broad variety)	9.4	1.893	.516	1.290

* It was only possible to carry the beet determinations over the first day because of a tendency to mold. The values here given were obtained by extrapolation.

No more need be said about the order of the vegetables with respect to the amount of CO_2 evolved than that it is in agreement with the general observation that the most rapidly developing tissue respires

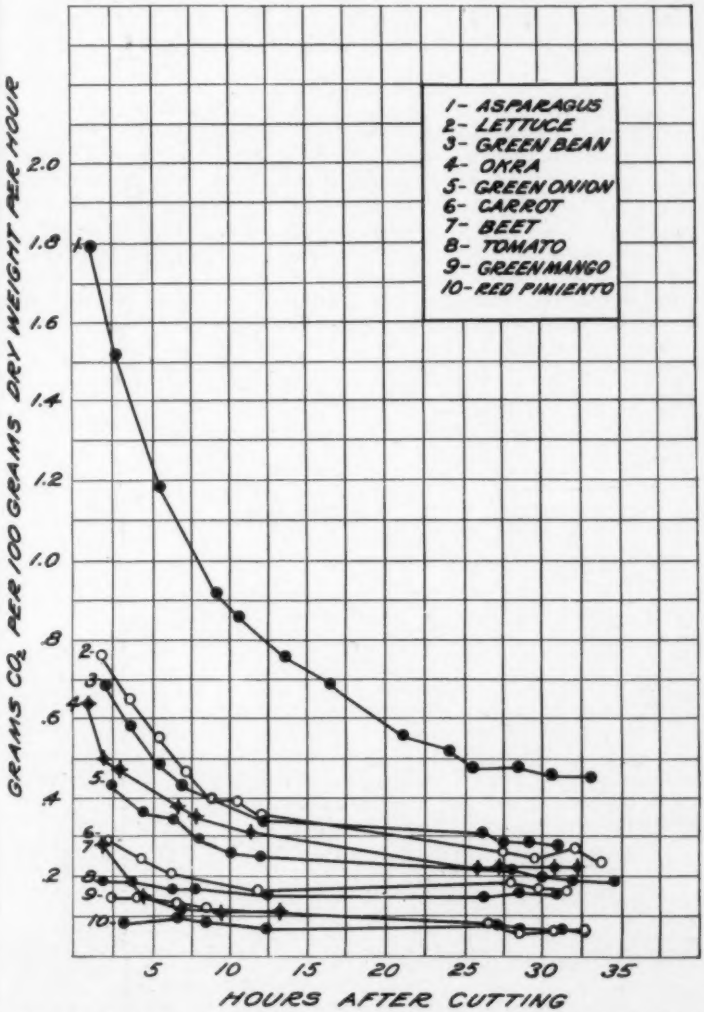

FIGURE 2.—Curves showing decline in respiration rate of fresh vegetables during the first 30 hours after cutting, when held at a constant temperature of 30° C. (±0.5)

most rapidly. That other significant changes, tending to decrease the food value of the vegetables under consideration, were occurring simultaneously is not to be doubted, such as, for example, an increase

in woody fiber.[6] This study presents only one phase—although probably the principal one—of the general deterioration process.

The vegetables came out of the thermostat (except the beet) after one and one-half days at 30° C., in an atmosphere nearly saturated with water vapor, looking as fresh and attractive as if they were straight from the garden—there was no outward indication of the changes which had gone on, except that the asparagus had increased a little in length.

SUMMARY

Ten green vegetables of edible maturity were subjected to comparative examination during the first 30 hours after they were harvested. Their changing rates of respiration at 30° C. were measured and plotted, the total amount of CO_2 evolved between the second and the twenty-sixth hours was calculated and the weights of glucose presumably oxidized in producing this quantity of CO_2 were computed. With respect to the amount of gas evolved for equal weights of dry matter during the 24-hour period, the vegetables stand in the following descending order: Asparagus, lettuce, green bean, okra, green onion, carrot, tomato, beet, green mango, red pimiento. The amounts of glucose accounted for vary from 13.682 gm. per hundred grams of dry weight in 24 hours for asparagus to 1.290 gm. for red pimientos.

[6] BITTING, K. G. DETERIORATION IN ASPARAGUS. Natl. Canners Assoc. Research Bul. 11, 18 p., illus. 1917.